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CLINICOHISTOLOGIC CLASSIFICATION OF THE GRAFT ILLNESS IN KERATOPLASTY

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Among various ophthalmic surgeries, keratoplasty still belongs to a group with higher postoperative complications. In 1948, Paufique, Sourdille and Offret[1] applied the term "Maladie du greffon" to the inexplicable development of necrosis and opacity in grafts implanted under normal conditions, with no manifest operative complication. Various interpretation have been given for the cause of pathological grafts. Frieberg[2], in 1914, tentatively interpreted as an anaphylactic reaction on his case with a virulent inflammation 14 days postoperatively. Later, Müller and Maumenee[3,4,5] emphasised the donor recipient reaction as the cause of graft opacification from their serial experimental evidences. On the other hand, Barraquer[6] pointed out the hypotrophy of the graft and he gave an attention on the nutrition of the graft. As the latest information, Hales and Spencer[7], in 1963, emphasized the wound margin abnormality which often developed the retrocorneal membrane by clinicohistologic observations on 40 cases of unsuccessful keratoplasty. They pointed out that the term "homograft reaction" to explain opacification of grafts due to clinically unknown causes has probably been overemphasized. Thus, differences of opinion concerning the cause of the graft illness seems to be due to a limited observation on the materials or experimental procedures selected by different investigators. Therefore, it is very necessary to analyse the cause of unsuccessful keratoplasty from clinical, pathological, physiological and biochemical view points by a single investigator at the same time. The purpose of this report is to classify the type of graft illness from a clinicohistologic entity.

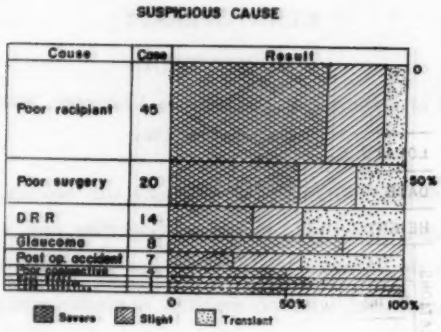
Clinical Analysis of the Graft Illness.

Incidence of pathological grafts: Among 114 eyes of penetrating keratoplasty, pathological condition of grafts appeared in 74 cases (64%). However, 20 cases had only transient graft illness and finally healed in transparent condition. Clinical analysis of the graft illness was done on these 74 cases.

Suspicious causes of pathological grafts (Fig. 1.): The cause of graft opacification was diagnosed by a high magnification slit-lamp, fluorescein diffusion test[8,9,10] and by clinicohistologic examinations mentioned in the next section. Among 74 eyes with graft illness, 105 causative events were estimated and classified as followes; unfavorable condition of reci-

This study was supported by the Fight for Sight Grant-in-aid (G233 C5) of the National Council to Combat Blindness, New York.

Fig. 1.



pient eyes was the most common cause and was found in 45 cases (43%). Incomplete surgery on microscopic level was 20 cases (17.5%). Homograft reaction was only 14 cases (12.2%), glaucoma 8 cases (7%), postoperative accident 7 cases (6.1%), poor conjunctival condition 4 cases (3.5%), poor donor material 3 cases (2.6%), recurrence of original disease 2 cases (1.8%) and late infection 2 cases (1.8%).

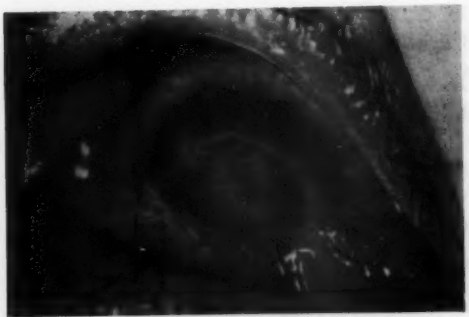
Clinicopathological Classification (Akagi and Tsutsui)

Up-to-date, reports on the clinical fate of the corneal grafts were explained by terms "transparent", "semitransparent" and "opaque". No histopathological consideration was given and causative factors were confused in the term "opaque graft". Clinicohistologic classification of "opaque graft" has been attempted on both clinical analysis on the above mentioned 74 eyes with graft illness and histopathological examination on 14 cases.

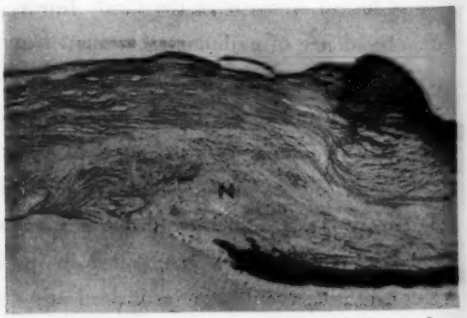
1. Atrophic type (Fig. 2).

Shrinkage and absorption of the graft which followed to the graft opacification was charac-

Fig. 2.



a. Chronic progressing atrophy of the graft. Inferior 1/4 of the graft is absorbed.



b. Acute progressing atrophy of the graft. Lyophilized cornea in penetrating keratoplasty.

terized by clinically. It must be due to the extensive disturbance of nutrition of the graft and complete loss of viability of the graft will be the direct mechanism. Partial necrosis without inflammatory cells, loss of staining ability by Eosin or PAS, and disappearance of keratocytes, endothelium and epithelium were the main histopathological changes.

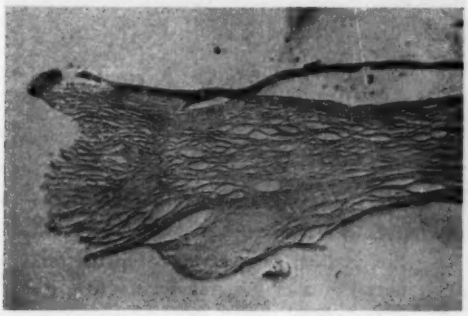
In this type, acute progressing atrophy and chronic progressing atrophy were subdivided. The acute type was only found in 2 exceptional cases of penetrating keratoplasty with a lyophilized graft and complete absorption of the graft developed within a month. The chronic type was occured in extensively damaged recipient eyes with the atrophy of intraocular tissue. These eyes often showed hypotonic intraocular pressure. The graft opacity started within the first postoperative week and very slow progressing atrophy of the graft followed during several months or a year. This type of graft illness was found in 7.8% of our penetrating keratoplasty (10.8% of the graft illness).

2. Dystrophic type (Fig. 3).

Clinical characteristics of the dystrophic type of the graft illness were opaque or semi-

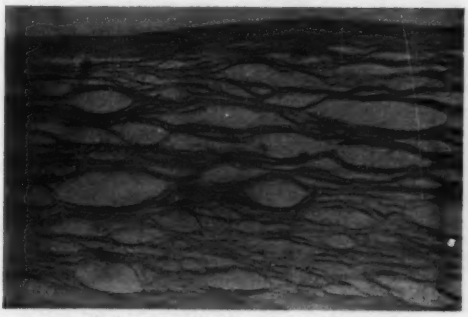
Fig. 3.

a. Dystrophic graft, clinical picture.



b. Posterior gap.

c. Bulla beneath the epithelium and edema in the stroma.

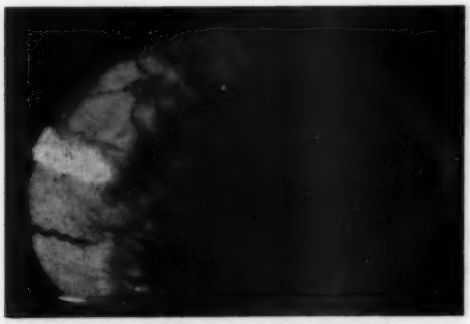


d. Acellular zone in the dystrophic graft.

b-d. Histopathology of dystrophic grafts.

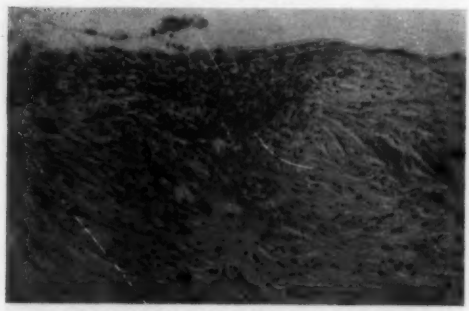
transparent graft with remarkable edema and bullous keratoplasty. No shrinkage of the graft developed within a few years. This type of the graft illness was recognized in 39.7% of the total cases (59.0% of pathological grafts) and was developed frequently in unfavorable indication of keratoplasty. Histopathological characteristics were the separation of lamella by an accumulation of water, decreased staining by Eosin or PAS, and decreased number of keratocytes. The endothelium was also degenerated or lost. There was little inflammatory cells or vascularization. The cause of dystrophic type was considered to be the failure of nutritional transport and disorder of water metabolism by the primary or secondary origin. The primary dystrophy was frequently developed during from the 20th to 40th postoperative days without any inflammatory sign. Disturbance of diffusion of fluorescein which was applied intravenously was often recognized in this type. The secondary dystrophy usually followed to the donor-recipient reaction or glaucoma. In these postoperative complications, distur-

Fig. 4. Donor-recipient reaction in the graft.

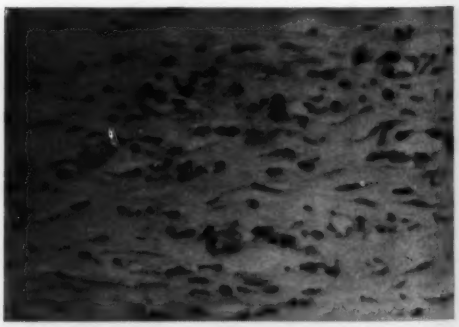


a. Precipitation lines appeared in the peripheral area of the graft on the first day of the onset.

b. 12 hours later, Precipitation lines moved to the center of the graft.



c. Histopathology of donor-recipient reaction around the coaptation area. Inflammatory cells infiltration.



d. Inflammatory cells and vascularization in the stroma. Neumerous eosinophil leucocytes were seen.

bance of nutritional transport was followed secondarily. Histopathological picture showed some inflammatory cells in the graft but graft edema which was characteristic in the dystrophic type was also apparent in the secondary dystrophy after inflammation.

3. Inflammatory type (Fig. 4).

Clinical peculiarities of this type was inflammatory findings in donor and recipient corneas; acute vascularization, acute progressing interstitial opacity, remarkable cicliary congestion, and iritis or iridocyclitis. The donor-recipient reaction was found in 12.2% of total cases (19.0% of the graft illness), infectious inflammation was in 2.6% (4.0%) and inflammation induced by radiation was in 1.7% (2.7%). In donor-recipient reaction, precipitation line of Wessery was recognized in the early stage (1st or 2nd day of the onset) by a careful slit-lamp examination and acute vascularization followed.

Histopathological characteristics of this type was the infiltration of numerous inflammatory cells (lymphocytes, eosinophyl- and neutrophyl leucocytes) in both the graft and recipient. In relatively late stage, numbers of fibroblasts and keratocytes increased extensively and cell rich connective tissue proliferated in the coaptation wound. This connective tissue may disturbe the diffusion track from the limbus to the graft and secondary dystrophy often developed after the inflammation had subsided.

4. Proliferating type

This type means a remarkable proliferation of abnormal tissue in and around the graft.

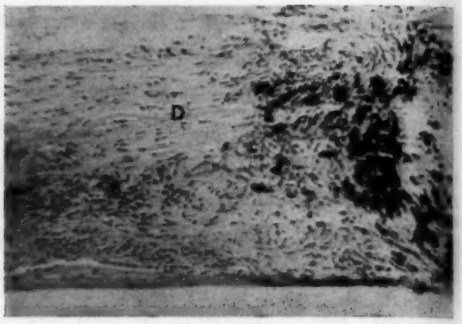
Granulation tissue: Elevated granulation tissue proliferated from limbar conjunctival keroid to the graft in one case of corneo-conjunctival heat burn.

Vascularization: Vascularization of grafts was found in 19.2% of total cases (19.7% of the graft illness) but extensive vascularization was only 1/3 of them. These cases were experienced before the advancement of strong steroid hormones. Two causative factors was recognized in the vascularization of grafts. One was inflammatory origin which usually followed to the donor-recipient reaction, while another was non-inflammatory origin which developed in the disturbance of nutritional transport especially in the graft surrounded by vascularized recipient cornea. Vascularization might be a protective mechanism for the nutritional disturbance and graft edema which usually developed in dystrophic type was never seen in a vascularized graft.

Retrocorneal membrane (Fig. 5): Halas and Spencer[7] emphasized the retrocorneal membrane in unsuccessful keratoplasty. In this clinical observation, retrocorneal membrane was found in 13.1% of the total cases (20.2% of the graft illness). Under a high magnified slit-lamp, white thin membrane was recognized on the posterior surface of the graft through a transparent or semitransparent graft. Histologically, this membrane was a proliferated connective tissue behind the Descemet's membrane from the recipient cornea at a coaptation

Fig. 5. Retro-corneal membrane of the graft.

a. Clinical picture.



b. Histopathology of the retro corneal membrane in inflammatory origine.

wound by both inflammatory and non-inflammatory origins.

Epithelial downgrowth (Fig. 6): Although epithelial downgrowth has been known in cataracta surgery, the histological evidence was occasionally demonstrated in a keratoplasty case with delayed wound healing.

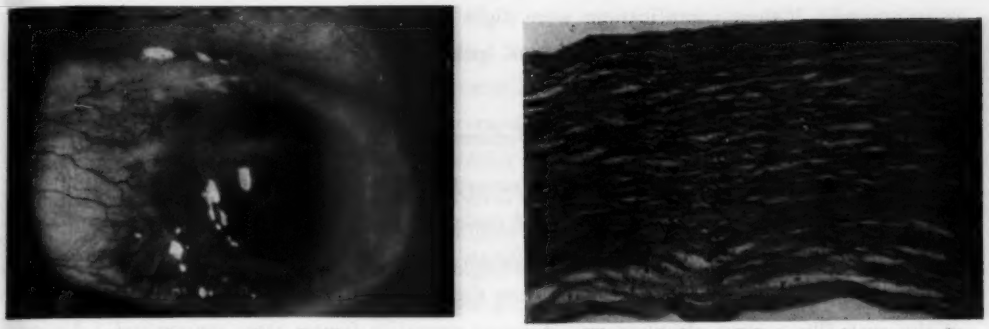
Fig. 6. Epithelial downgrowth at the margine of the graft.

5. Necrotic type (Fig. 7).

Clinical characteristic of this type was graft necrosis like keratomalacia without an infection. This type was only seen in one case which had a radiation treatment (^{90}Sr, 10,000 rep.) 3 months before the keratoplasty. Histologic characteristics were the defect of epithelium and endothelium, slight edema of the stroma, disappearance of ground substance (PAS) between collagen bundles and no inflammatory cell infiltration. The cause of this type was thought to be an intensive metabolic disorder, especially the synthesis of mucoprotein and seemed to be more intensive form of the dystrophic type.

6. Relapse of original disease in the graft.

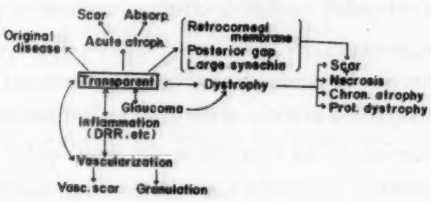
Fig. 7.



a. Necrotic type of graft illness. b. Histopathology of the necrotic type graft.

Fig. 8.

COURSE OF THE GRAFT ILLNESS



Relapse of Groenouw's dystrophy was experienced in two cases (2.8% of the graft illness) 6 and 13 months postoperatively. Relapse of herpetic keratitis was not experienced in this series.

7. Scar type.

Primary scar developed in the coaptation wound was non-pathologic character. Pathological scar tissue was developed finally after various types of graft illness such as dystrophic type, inflammatory type and proliferative type. The clinical picture showed hard corneal opacity which was constructed by irregularly arranged fibrous tissue in histology. There was no edematous opacity like dystrophic type. Epithelial papilla, irregular thickness of the epithelum, irregular lamella, increased keratocytes, reduction of polysaccharides, vascularization with perivascular connective tissue and degenerative changes (calcification or fat deposite) were also peculiar.

8. Course of pathological grafts.

Since various pathogenesis influenced a development of graft illness, the course of pathological grafts were relatively complicated. As illustrated in Fig. 8, grafts getting a transparent healing became cloudy by various factors. Among them, graft illness caused by inflammation,

glaucoma, vascular proliferation and dystrophy could be recovered from opaque condition to transparent, if these complications were slight or short duration. The acute and chronic progressing atrophy, retrocorneal membrane, granulation, necrosis, and relapsus of original disease were non-reversible change.

Discussion

The results in this investigation were obtained from 114 cases of penetrating keratoplasty which were performed during 1957 to 1964 under a routine postoperative management with steroid medication. To analyse the graft illness and to improve the treatment, keratoplasty was performed under wide indication. From the clinicohistologic observation, various types of the graft illness were classified and different causative factors were recognized. Among all sorts of the graft illness, dystrophic type were the most common and the most important. Histopathological characteristic is edematous swelling of the graft. Biochemical peculiarity is depressed metabolic activity[11] which developed by an incomplete transport of the nutrient from the recipient eye. Dystrophic change is often occurred in a poor recipient eye and in unproper coaptation of the wound. In these cases, definite disturbance of diffusion from the recipient cornea to the graft can be demonstrated by fluorescein test[11]. From the therapeutic view point, to increase the metabolic activity of the graft by instillation of metabolic accelerators may result a rapid consumption of the nutrient in the graft under incomplete transport and clinical results shows no benefit. Excessive topical use of steroid hormone also brings a harmful result in dystrophic graft, because steroid hormones are metabolic inhibitor and depress the asthenic metabolism of pathological grafts.

Graft illness due to homograft reaction are completely different from the dystrophic graft in histopathological characteristics. Under a good understanding of clinicohistologic characteristics of allergic graft illness, clinical diagnosis of the donor-recipient reaction is relatively easy. As Hales and Spencer[7] pointed out, the homograft reaction has been overemphasized in keratoplasty. By the development of anti-allergic drugs, this type of the graft illness has been and will be reduced more. In this type, graft metabolism is accelerated extensively differently from the dystrophic type. Clinical diagnosis of unsuccessful keratoplasty must be considered on clinicopathological entity.

Conclusion

The purpose of this study is to clarify the pathogenesis of unsuccessful keratoplasty from clinicopathological entity.

On the clinical statistics in 114 cases of penetrating keratoplasty, illness of the graft was found in 74 eyes (64%) and 54 eyes out of 74 developed permanent pathology of the grafts. On suspicious causes of pathologic grafts, hypotrophic condition due to unfavorable recipi-

ent eyes or a microsurgical error was the most common (61%) while donor-recipient reaction was minor (13%).

Seven types of graft illness were classified from the clinical and pathological points of view. They were atrophic type (acute and chronic), dystrophic type (primary and secondary), inflammatory type (includes donor-recipient reaction), proliferative type (gramulation, vascularization, retrocorneal membrane and epithelial downgrowth), necrotic type, recurrence of original disease and scar type. Histopathological alteration (edema, reduction of mucoid and acellular zone) in dystrophic grafts were different from those in grafts with donor-recipient reaction (Arthus type). For the clinical diagnosis of unsuccessful keratoplasty, clinicopathological entity must be attached great importance.

REFERENCES

1. Paufique, L., Sourdille, G.P. and Offret, G.: Les greffes de la cornee, Paris, Masson and Cie, 1948, p. 258.
2. Frieberg, T.: Klin. Monatsbl. Aug., 52:436, 1914.
3. Müller, H. and Maumenee, E.A.: Arch. f. Ophth., 152:1, 1951.
4. Maumenee, A.E.: Amer. J. Ophthal., 34:II, 142, 1951.
5. Maumenee, A.E.: Tr. Amer. Acad. Ophthal. Otol., 64:765, 1960.
6. Barraquer, J.I.Tr.: Amer. J. Ophthal., 33:II, 6, 1950.
7. Hales, R.H. and Spencer, W.H.: AMA Arch. Ophthal., 70:805, 1963.
8. Tsutsui, J., Honda, N., Kobashi, T., Nobuto, F., Watanabe, S. and Yamori K.: Acta Soc. Ophth. Jap., 68:946, 1964.
9. Tsutsui, J.: Fol. Ophth. Jap., 16:515, 1965.
10. Honda, N.: Fol. Ophth. Jap., 16:611, 1965.
11. Tsutsui, J.: Acta Soc. Ophth. Jap., 69:1814, 1965.

EXPERIMENTAL STUDIES ON THE CORNEAL DAMAGE BY THE OPHTHALMIC OINTMENT AND OIL

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Ophthalmic ointment and oil have been employed by opthalmologists universally for their two charactristics, one is long remaining in the cul de sac and other is the good penetration into the ocular tissue.

However the experiment about the corneal damage, especially about the influence on the regeneration of the corneal wound by ointment are scarce.

In this experiment glycogen staining and alkaline phosphatase reaktion in the corneal tissue have been observed from the point of corneal metabolism for the purpose to estimate

the damage by various ointments and oils both to the normal cornea and artificially injured cornea.

Experiment 1. Change of glycogen staining in the rabbits cornea by ophthalmic ointments and oils.

Material and Method

The bases employed in this experiment are 16 kinds in all. (Table 1)

Both ointments and oils have been applied 3 times and 1 time daily respectively in the cul de sac of rabbits, and then tarsorrhaphy was performed only with the right eye.

The artificial wound has been made by the double parallel incision of the corneal surface vertically with the distance of 2 mm and then the epithelium within the incision was completely abraded. The eyeballs was enucleated 24 hours after for the staining. The bases of ophthalmic ointment used in this experiment are listed in table 1.

Table I. Ophthalmic Ointment Bases

1. Hydrophilic Bases	White Petrolatum
Propeto+Wool Fat (10%)	Propeto
Hydrophilic Petrolatum	Plastibase
Carbowax 1500+Purified Water	3. Oil
Wool Fat	Liquid Paraffin
Yellow Petrolatum+Tween 20 (1%)	Camellia Oil
Camellia Oil+Tween 20 (1%)	Salad Oil
Propeto+Tween 20 (1%)	Sesame Oil
2. Hydrophobic Bases	Camellia Oil+Aluminum
Yellow Petrolatum	Monostearate (2%)

Fixations method: The resected corneas are fixed in ice acetate (4°C) for 2 hours and paraffin sections were prepared. Some of the corneas were fixed in Carnoy's solution for 30 minutes.

Staining: Both the hematoxylin-eosin stain and Periodic Acid Schiff reaction were performed.

Results

1. Changes in normal cornea.

A. *Hydrophobic bases.*

Yellow vaseline, white vaseline, propeto (one of the pure white vaseline) and plastibase are used.

Histological findings:

The most marked damage was observed with white vaseline and the least one was plastibase. By the application of white vaseline degeneration and necrosis have been observed in

the central region of the corneal epithelium but the emigration of monocytes and polymorphnuclear cells and the growth of connective tissue were recognized under the necrotized epithelium. On the other hand the corneal epithelium has showed none of the regressive findings after the application of plastibase.

Glycogen staining:

The grade of glycogen staining in the corneal epithelium were well reflected to the histological finding.

Glycogen has disappeared at the central region of the corneal epithelium after the application of a white vaseline and he PAS reaction has been recognized only in the deep layre of the corneal stroma.

On the other hand the grade of glycogen staining was almost the same with the normal control in case of plastibase.

B. *Hydrophilic bases.*

Propeto and lanolin (10%) base, propeto and tween 20 (1%) base, camellia oil and tween 20 (1%) base and carbowax were used in this experiment.

Figs. 1-4. Damage of the normal cornea after the application of various ophthalmic ointments. Stained with hematoxylin and eosin. 50×

Fig. 1. Normal corena.

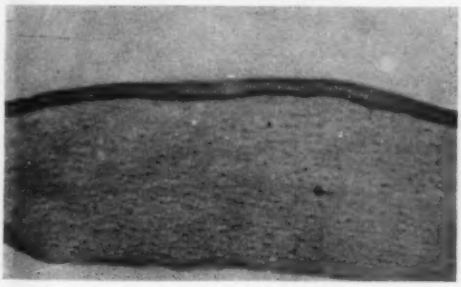


Fig. 2. Liquid paraffin.

Fig. 3. Propeto.

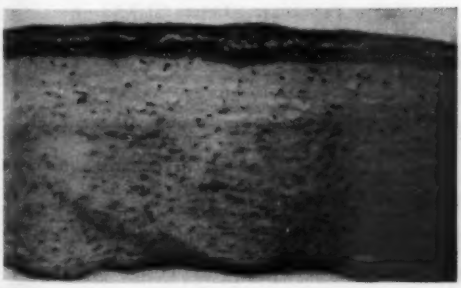


Fig. 4. Lanoline.

Histological findings:

The marked damages were observed by all of the hydrophilic bases employed in this experiment.

Glycogen staining:

Glycogen has diminished or disappeared in the corneal epithelium by all bases of hydrophilic type.

C. *Oil bases.*

Liquid paraffin, camellia oil, salad oil and sesame oil are used as ophthalmic oil bases.

Histological findings:

Vacuolar degeneration was observed in the middle and deep layers of the epithelium and the growth of connective tissue are marked in the stroma.

Glycogen staining:

Glycogen has disappeared partly in the epithelium and also diminished at the basement of the epithelium. (Table II, III).

Table II. Hematoxylin—Eosin Staining of the Normal Cornea

Bases	Epithel Necrosis	Degeneration	Stroma		
			Monocyte	Leucocyte	Connective tissue
Yellow p.	−	+	+	−	+
White P.	+	++	++	++	++
Propeto	−	++	+	−	+
Plastibase	−	−	+	−	±
Propeto+Wool Fat (10%)	−	++	++	++	+
Wool Fat	+	+++	+	−	+
Yellow P.+Tween 20 (1%)	±	++	±	−	+
Hydrophilic P.	+	+++	±	−	+
Liquid Paraffin	−	++	+	−	+

Table III. Glycogen Staining of the Corneal Epithelium (Normal)

Yellow Petrolatum	+	Wool Fat	−
White Petrolatum	−	Yellow Petrolatum+Tween 20 (1%)	−
Propeto	++	Liquide Paraffin	+
Plastibase	+++	Control	+++
Propeto+Wool Fat(10%)	−		

II. Changes in artificially injured corneas.

A. *Hydrophobic base.*

Histological findings: Erosions on the surface of the wound and vacuolar degeneration around the wound were observed by the application of propeto, but these changes were absent

Figs. 5-8. Damage of the normal cornea after the application of various ophthalmic ointments. PAS stain.

Fig. 5. Normal corena.

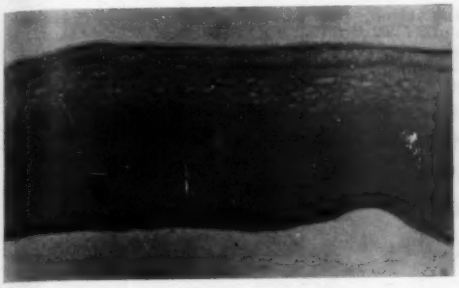


Fig. 6. Propeto.

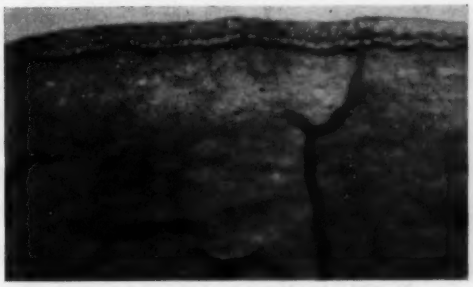
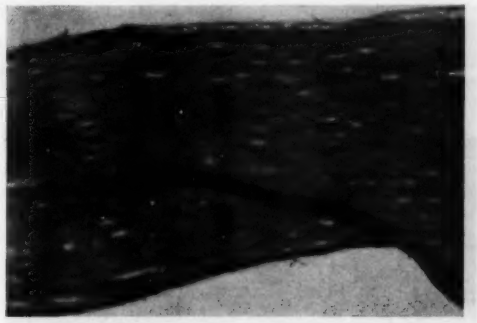


Fig. 7. Lanolin.

Fig. 8. Yellow petroladum and tween 20 (1%).



in case of plastibase. The emigration of monocytes and polymorphnuclear cells and the growth of the connective tissues have been observed in the stroma under the wound by the application of all bases.

Glycogen staining:

Glycogen was markedly diminished in the regenerated epithelium over the wound in case of propeto but the change was slight in the application of plastibase.

B. *Hydrophilic bases.*

Histological findings:

Vacuolar degeneration was marked in the middle and deep layers of the regenerated epithelium over the wound and the cell arrangement were also irregular by the application of lanolin and propeto base.

These damage was somewhat slight in case of propeto and tween base.

Glycogen stainings:

The diminution of glycogen staining in the regenerated epithelium by the application

Table IV. Hematoxylin-Eosin Staining of the Injured Cornea

Bases	Injured Cornea						Control					
	Epithel		Inj. Part				Epithel		Inj. Part			
	Nec.	Deg.	Deg.	Mon.	Leuc.	Conn.	Nec.	Deg.	Deg.	mon.	Leuc.	Conn.
Propeto	−	+	−	+	+	+	−	−	−	+	++	+
Propeto (48st)	−	+	−	+	++	+	−	−	−	++	++	++
Plastibase	−	−	−	++	++	++	−	−	−	+	++	+
Propeto+Wool Fat (10%)	−	++	+	++	++	+	−	−	−	+	++	+
Propeto+Tween 20 (1%)	−	+	±	+	+	++	−	−	−	+	+	+
Camellia Oil+Tween 20 (1%)	−	+	+	++	++	+	−	−	−	+	+	+
Carbowax 1500+Purified Water	−	+	±	+	+	±	−	−	−	+	+	+
Camellia Oil+Al. Monost. (2%)	−	±	−	+	+	+	−	−	−	+	++	+
Camellia Oil	−	±	±	+	±	±	−	−	−	+	+	+
Salad Oil	−	−	−	+	+	+	−	−	−	+	+	+

Figs. 9-11. Damage of the artificially injured cornea after the application of various ophthalmic ointments. PAS stain.

Fig. 9. Propeto.

Fig. 10. Camellia oil and tween 20 (1%).

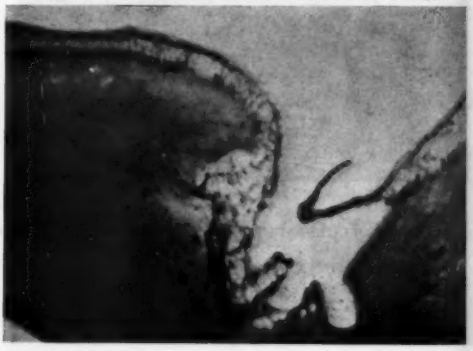


Fig. 11. Camellia oil.

of lanolin base has been more marked than propeto and tween base.

C. *Oil bases.*

Histological findings:

Vacuolar degeneration was observed slightly only by camellia oil. The most harmless base is salad oil.

Glycogen staining:

The diminution of glycogen staining was observed by camellia oil in the regenerated epithelium over the wound and the epithelium near the wound. In case of salad oil the grade of staining was almost same to the control. (Table IV)

Experiment 2. Change of alkaline phosphatase reaciton in the rabbits cornea by ophthalmic ointments and oils.

Material: The same as experiment I.

Method: Takeuchi's method is used as staining.

The resected cornea is fixed in ice acetate and dehydrated for 2 hours and then paraffin sections are prepared. After deparaffinization by benzine the preparations is kept in the substrate solution for 3 hours at 37°C.

Substrate solution is as follows.

1% Natrium glycerophosphate	10.0
1% Calcium chloride	10.0
buffers	5.0
5% Magnesium chloride	2.5

After washing kept in 3% silber nitrol solution for 10 minutes exposing under the direct sunlight.

Washing again and then kept in 3% sodium thiosulphate solution for several minutes.

Results

I. Changes in normal cornea.

A. *Hydrophobic bases.*

At the central region of the cornea the reaction was negative from epithelium to endothel, only showing the slight staining at the limbus. Plastibase and propeto have caused, however, the least effect compared with control.

B. *Hydrophilic bases.*

Falling-off of the epithelium was marked with lanolin base and pharmacopoeial hydrophilic ointment base only showing obvious reaction at the remaining epithelium. Almost same effects were observed by lanolin propeto and tween propeto bases.

C. *Oil bases.*

Liquid paraffin has caused slightly diminished activity compared with control but the

Table V. Al. Phosphatase Reaction of the Normal Cornea

Bases	Treated Eye				Control			
	Surface of Epithel (1)	Epithel (2)	Stroma (3)	Endoth. (4)	(1)	(2)	(3)	(4)
Yewllow P.	+	−	−	−	++	±	−	−
White P.	−	−	−	−	++	±	−	±
Propeto	+	±	−	−	+++	++	+	±
Plastibase	++	++	−	−	++	++	−	−
Propeto+Wool Fat (10%)	−	−	±	±	++	++	+	+
Wool Fat	−	−	−	−	++	±	−	−
Yellow P.+Tween 20 (1%)	±	±	−	−	+	±	−	−
Hydrophilic P.	−	−	+	±	++	+	±	±
Liquid Paraffin	++	+	±	−	++	++	±	±

Figs. 12-15 Damage of the normal cornea after the application of various ophthalmic ointments. Alkaline phosphatase reaction.

Fig. 12. Normal cornea.

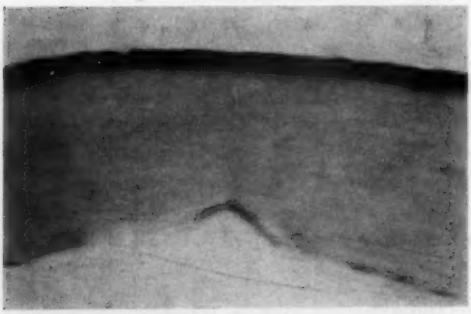


Fig. 13. Plastibase.

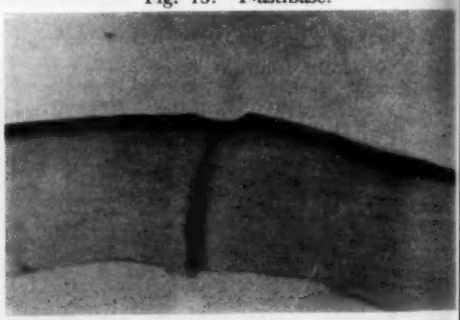


Fig. 14. Propeto.

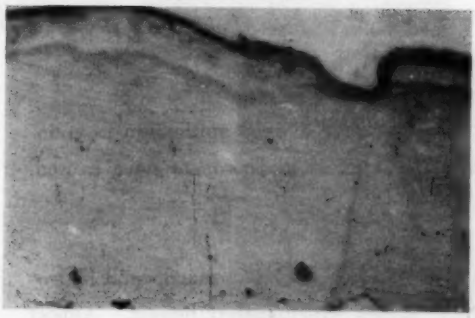


Fig. 15. Propeto and lanolin (10%).

effect was slight next to plastibase and propeto. (Table V)

II. Changes in artificialy injured corneas.

A. *Hydrophobic bases.*

Absence of regenerated epithelium over the wound and a low activity at the peripheral part of cornea were observed by the application of propeto, but no changes were observed in case of plastibase.

B. *Hydrophobic bases.*

Low activity at the epithelium and stroma were obvious by all kind of hydrophobic bases.

C. *Oil bases.*

The lowering of activity was not marked in case of oil bases.

Figs. 16-18 Damage of the artificially injured cornea after the application of various ophthalmic ointments. Alkaline phosphatase reaction.

Fig. 16. Propeto (48 hours).

Fig. 17. Salad oil.

Fig. 18. Camelia oil and tween 20 (1%).

Discussion

Change of glycogen by the application of ointments and oils.

Respiration and glycolysis are most important metabolism of the cornea and air oxygen is thought to be one of a inevitable source of corneal respiration[1,2,3]. The application of ophthalmic ointments and oils are, therefore, seemed to give some effect to the metabolism

of corneal tissues. In the fact, necrosis and vacuolar degeneration of the corneal epithelium have been observed histologically more or less by the application of all the type of ointment bases in this experiment.

The most marked damage has been caused by hydrophilic bases and the weakest was oil bases. In hydrophobic bases plastibase was the most harmless base, but a white vaseline has been unexpectedly more stimulative than a yellow vaseline. The damage was caused slightly in the artificially injured corneal wound as compared with normal, and this is seemed to depend on the increase of corneal metabolism for the wound healing. The change of glycogen staining by the application of ointment bases has been almost coincided to the histological findings and slightly diminished only in the case of artificially injured wounds.

The diminution or disappearance of glycogen in the corneal epithelium suggests the increase of glycolysis in that tissue to repair the damage caused by the application of ointments and oils which inhibits the corneal metabolism both from air oxygen and limbal blood circulation.

On the other hand in the artificially injured corneal wound the increase of glycogen has been observed both at the corneal stroma beneath the epithelium where the glycogen staining disappeared or diminished and in the emigrated leucocytes in stroma. These increase of staining has been also observed at the regenerated epithelium over the wound and this findings is thought to imply the starting of regenerations process which requires the storage of glycogen.

Change in alkaliphosphatase activity.

Since alkaliphosphatase has a important relation to the corneal metabolism, it may be conceivable that the application of ointment and oil bases will give some effect to the alkaliphosphatase activity of the cornea.

Kubo[4] has reported that the alkaliphosphatase activity reduces or disappears both in the degenerative change of cells and in necrotic tissues.

The same lowering of activity has been proved by Noda[5] in the experiment of corneal damage used chemical agents. The lowering or absence of activity have been also observed by the application of many type of ointment bases in my experiment and it may be thought to suggest the reduction of corneal metabolism as the result of damage by ointment. The most harmful bases were hydrophilic bases and white vaseline among bases employed in my experiment. These bases have caused the falling-off of the central region of epithelium or lowering of the activity in the all layers of epithelium.

On the other hand, there have observed relatively slight lowering of activity by the application of hydrophobic bases or oils. This may be due to the loose connection to the corneal surface, lower viscosity and short remaining time in the cul de sac of these bases.

The effect on the activity is not severe in the artificially injured cornea compared with

normal and this is due to the increase of enzimic activity by the injury. This increase of activity may be also thought as the representation of a protective reaction of the cornea. However the reduction of the alkalinephosphatase activity of some extent observed in the experiment of normal and injured cornea may be imply that the ointment or oil bases have had much or less harmful effect on the corneal metabolism.

Conclusion

Corneal damage after the application of various ophthalmic ointments and oils was investigated histochemically by hematoxyline-eosin, glycogen and alkalinephosphatase staining with normal and artificially injured corneas.

1) None of sixteen kinds of agents were effectless to the corneal epithelium, but the agent which showed the most harmful damage was hydrophilic ointments.

2) The diminution of corneal glycogen and the low activity of alkalinephosphatase in the cornea were observed by the application of agent parallel to the grade of damage in order of hydrophilic, hydrophobic ointment and oil.

3) The damage observed by glycogen and alkalinephosphatase staining was less marked in artificially injured cornea than the normal cornea, but it seemed to be manifest that all kinds of agents acted harmfully to the regeneration of corneal wounds.

REFERENCES

1. Kohra, T.: Acta Soc. Ophth. Jap. 40:1993, 1936.
2. Simoyama, K.: Acta Soc. Ophth. Jap. 45:1931, 1941.
3. Langham, M.: J. Physial., 177:461, 1952.
4. Kubo, H.: Kumamoto Igakkai-Zasshi 7:2, 2551, 1954.
5. Noda, T.: Acta Soc. Ophth. Jap. 58:651, 1954.

CLINICAL STUDIES ON CENTRAL SEROUS CHORIORETINITIS BY FLUORESCEIN FUNDUS PHOTOGRAPHY

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Novotny and Alvis[7] described a new method for the study of retinal blood flow in man by the use of intravenous fluorescein in retinal photography and this technique promised to reveal intra vitam some of the fine details previously seen only in necropsy studies.

The purpose of this paper is to present the fluorescein fundus photographs of central serous chorioretinitis (Masuda), and to discuss the clinical and pathological features of this disease.

The results of the studies have been already stated in details by the author at the Japanese Ophthalmological Society (April, 1965)[2].

Method and Material

For the author's investigation, the fundus camera of Carl Zeiss Jena was modified in order to elucidate the mode of passage of fluorescein via retinal circulation in photographs and the device was basically the same as the apparatus described by Novotny and Alvis. The camera was equipped originally with an electric flash, the maximum discharge of which allowed one photograph every 4 seconds. Tri-X Pan 35 mm film (Kodak) was used; it was force-developed for 30 minutes at 18 °C with Konidol Super developer (Konishiroku Photo Ind. Co., Ltd.).

Six normal subjects and 23 patients with central serous chorioretinitis (Masuda) were studied by the fluorescein fundus photography. In seven patients of them, the clinical courses of this disease were followed for long periods of time using the fluorescein method, and some albino rabbits were also observed in order to clarify the role of the pigment epithelial layer in transmission of fluorescence from the choroidal circulation.

Results

Normal subjects.

The time from injection into the antecubital vein until visualization of fluorescence in fundus photograph varied from 10 to 20 seconds. The arteriolar, capillary and venous phases in retinal circulation could be identified in the serial photographs. In the early venous phase, the appearance of the laminar flow reported by Novotny and Alvis was confirmed. In the

Fig. 1-A (Normal fundus). Picture taken 27 seconds after 5 cc of 5 per cent fluorescein was injected intravenously. The arrow points to laminar flow in the vein.

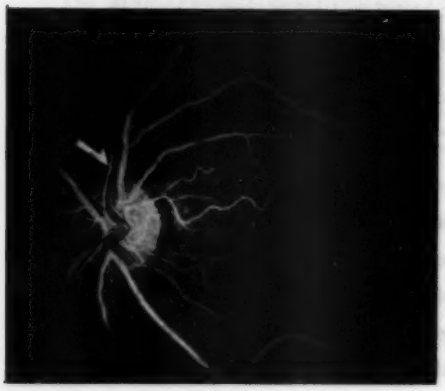
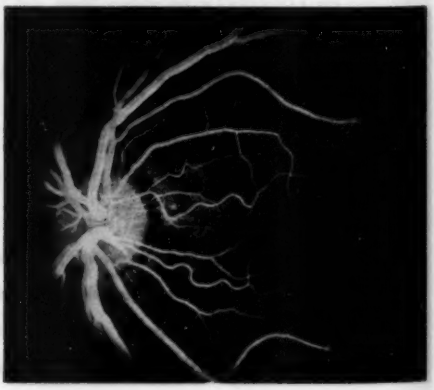


Fig. 1-B (Same eye as Fig. 1-A). Picture taken 38 seconds after injection of the dye. The venous filling phase.

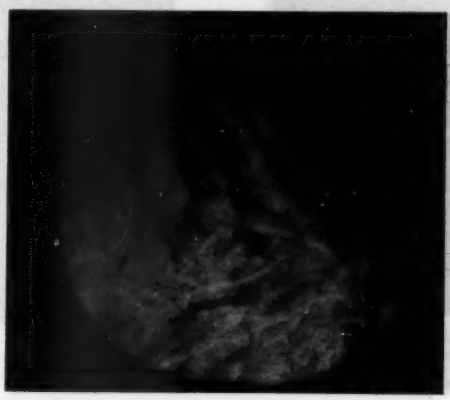


capillary phase, individual capillaries could be identified on some of the best photographs. The foveolar region remained dark in contrast to the rest of the fundus (Figs. 1-A, 1-B).

Albino rabbits.

The choroidal vessels as well as the retinal ones fluoresced about 5 seconds after injection of the dye (Fig. 2).

Fig. 2 (Albino rabbit). The choroidal vessels and some of the retinal arteries are filled with the dye 5 seconds after intravenous injection of fluorescein.



Patients with central serous chorioretinitis.

In all of 23 cases abnormal findings were observed by the fluorescein fundus photography. The typical cases were presented as follows:

Case I.

Man aged 46 with 7-days history of central scotoma and micropsia in the right eye was observed. The retina was turbid and slightly elevated three disc diameters in size at the posterior pole, where a white-yellowish fleck was detected by careful examination with the direct ophthalmoscope. Another smaller disciform lesion was found temporal to the macula lesion and the retina thereof was distinctly elevated and appeared a little reddish (Fig. 3-A).

Intravenous fluorescein revealed a small fluorescent spot in the macular region and this spot was increased markedly in intensity and size for subsequent few minutes without fluorescence of the remaining elevated retinal area. This fluorescent spot accurately corresponded to the ophthalmoscopical white-yellowish fleck. On the other hand, the entire area of the retinal elevation which situated in the temporal side of the macular lesion was permeated by the dye alredy at the arterio-venous phase. This fluorescent area did not change in size, but was increased in intensity of fluorescence after its first appearance (Figs. 3-B, 3-C).

Twenty-five days after the first examination, these two lesions were found to move slight-

Fig. 3-A (Right eye in Case I). Ordinay fundus photograph. The white arrow in the upper portion indicates small disciform area of the elevated retina and the lower one points to large disciform macular lesion. A black arrow indicases a white-yellowish fleck within the large macular lesion.

Fig. 3-B (Same eye as Fig. 3-A). Fluorescein photograph taken 57 seconds after intravenous injection. Visibility of the retinal vessels is partially obscured by fluorescein permeated into the entire area in the lesion temporal to the macula. An arrow indicates fluorescent spot corresponding to the black arrow in Fig. 3-A.

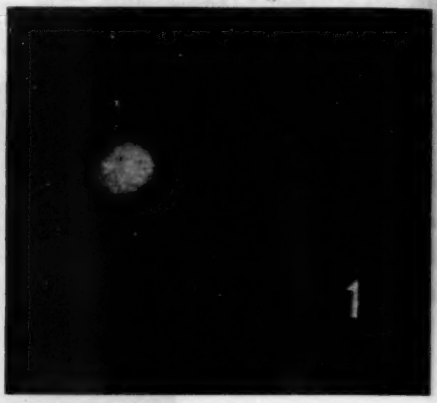


Fig. 3-C (Same eye as Fig. 3-A). Two and one-half minutes after intravenous injection. A fluorescent spot which indicated by the white arrow in Fig. 3-B spreads over with time.

ly downwards, the retinal elevation being decreased in size and degree. At this time, intravenous fluorescein revealed small fluorescent spots which corresponded to the ophthalmoscopical white-yellowish fleck as it had been, and these fluorescent spots spreaded for subsequent few minutes. On the other hand, fluorescence was found in entire lesion of the elevated retina situated in the temporal side of the macular lesion, the fluorescence, however, being more faint than that observed in the first examination (Figs. 4-A, 4-B).

Fig. 4-A (Same eye as Fig. 3-A). Fluoresein picture taken 25 days after the first fluorescein fundus photography. Note the small fluorescent spots in the area corresponding to that indicated by the arrow in Fig. 3-B.

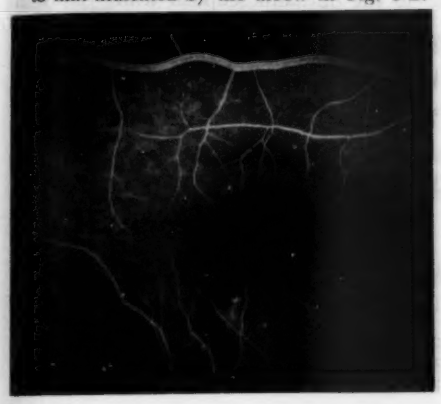
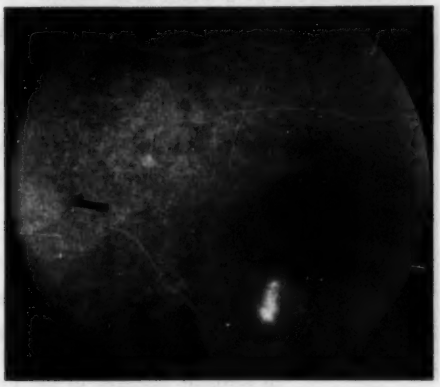


Fig. 4-B (Same eye as Fig. 4-A). Two minutes later. The small fluorescent spots are increased in intensity and size. The arrow points to faint fluorescence in the disciform lesion temporal to the macula.



Case II.

This case was a man aged 43, with complaint of visual disturbance in the right eye and was first examined 20 days after the onset. On examination with the direct ophthalmoscope, a serous detachment of the retina was found in the macula, which showed an area two disc diameters in size and was not so well-defined. A white-yellowish fleck was detected in the

Fig. 5-A (Right eye in Case II). Ordinary fundus photograph. The arrow points to a white-yellowish fleck in the upper and temporal margin of the disciform elevated lesion in the macula.

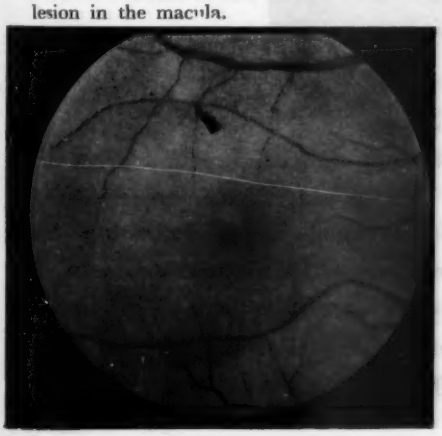
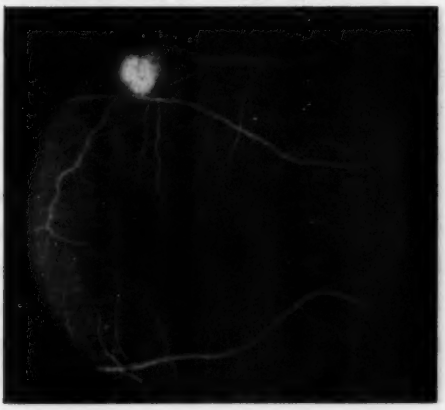


Fig. 5-B (Same eye as Fig. 5-A). Picture taken three minutes after injection of fluorescein. Note a fluorescent spot corresponding to the arrow in Fig. 5-A.

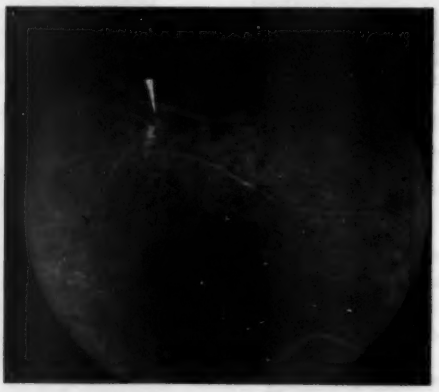


margin of disciform elevation of the retina only by careful examination with the direct ophthalmoscopy and it was about one-fourth disc diameter in size (Fig. 5-A).

When the patient was given fluorescein intravenously, the entire area of detachment did not fluoresce, but a small area which corresponded to the opthalmoscopical white-yellowish fleck diffusely fluoresced and then spreaded slowly after its first transit (Fig. 5-B).

Nine months after the first examination, the patient had neither visual disturbance nor abnormal findings in the fundus. Intravenous fluorescein, however, produced slightly abnormal fluorescent spots which did not spread after its first appearance, and these small spots were located within the area where diffuse fluorescence had been observed at the first examination (Fig. 6).

Fig. 6 (Same eye as Fig. 5-A). Fluorescein picture taken nine months after the first fluorescein photography. Note the small fluorescent spots wthin the area corresponding to the arrow in Fig. 5-A. These spots indicates underlying choroidal fluorescence which is transmitted because of atrophic changes in the pigment epithelium (Arrow).



Discussion

Normal pattern of fluorescein fundus photography.

The time from injection of the dye until visualization of fluorescence ranges from 10 to 20 seconds, and the result is almost the same as that reported by other investigators[1,7,8]. The foveolar region does not fluoresce, because this region is avascular and regardless of the thinnest part of the retina, transmission of fluorescence from the choroidal circulation seems to be prevented by the pigment epithelial layer acting as a filter to fluorescence. This opinion is supported by the results of studies in albino rabbits, in which the pigment epithelium contains little or no melanin. Additionally, in normal subjects (Japanese) the conspicuous "choroidal flush" can not be seen in almost all cases.

Fluorescence pattern in cases with central serous chorioretinitis.

When examined with fluorescein method, all of 23 cases have shown more or less abnormal findings. These abnormal findings produced by intravenous fluorescein can be classified into two types.

In the first type of fluorescence, the entire area of the elevated retina is permeated by the dye. This type of fluorescence is found in only two of all cases, and seems to be observed only in early stage of this disease. The dye in the entire lesion permeates with time into the inner retinal layer and the retinal vessels may come to be covered by the dye (Fig. 3-B). This fact shows that the fluid containing fluorescein not only remains in the subretinal space, but also permeates into the retina therefrom.

In the second type of fluorescence, intravenous fluorescein reveals a small area of fluorescence and this fluorescent spot is markedly increased in intensity and size for subsequent few minutes and corresponds to the ophthalmoscopical white-yellowish fleck. The similar fleck has been reported to be detected in about 70% of cases with central serous chorioretinitis specifically by careful examination with the direct ophthalmoscope[4]. It has been considered by many Japanese investigators that the white-yellowish fleck is the evidence of choroidal lesion[4,6]. From the results of the author's observations, it is most likely that such a fluorescent spot appears to be due to a secondary response to a focal lesion in the choroid which results in an increased permeability of the involved choriocapillaris and leads to secondary functional or organic disturbances of the pigment epithelial layer. There is no evidence of a changed retinal vascular permeability in all of the cases.

In the clinical course of this disease, there may be occasionally small pigmentary defect resembling a hyalin body of the choroid on examination with the ophthalmoscope, and by intravenous fluorescein, small fluorescent spot is observed in the area corresponding to pigmentary disturbance during the early arterial phase and persists as long as enough circulating fluorescein. Even when ophthalmoscopical abnormal findings completely disappear with healing of this disease, such a fluorescent spot may be detected. This fluorescent spot is well-defined and do not change its size with the course of time, and this would seem to be related in some way to the changes in the overlying filter - atrophic lesion in the pigment epithelium. Thus, the underlying choroidal fluorescence is readily seen.

From above-mentioned results of intravenous fluorescein studies, the descriptive name "central angiospastic retinitis (Hornicker[5]) or central angiospastic retinopathy (Gifford and Marquardt[2]) for central serous chorioretinitis (Masuda[6]) is not appropriate, if the macular lesions denoted by these different names are of the same entity from the clinical and pathological points of view.

Summary

1) By intravenous fluorescein, the arteriolar, capillary and venous phases in normal retinal circulation can be identified, and normal fluorescence pattern is discussed.

2) Abnormal fluorescence findings observed in all of 23 cases with central serous chorioretinitis are reported and discussed. From the observation of the clinical course of this disease, it is clarified that some different types of fluorescence pattern can be identified. These results suggest that the basic process of central serous chorioretinitis lies in the choroid.

REFERENCES

1. Dollery, C.T., Hodge, T.V., and Engel, M.: Brit. Med. J., 5270:1210, 1962.
2. Fujisawa, Y.: Acta Soc. Ophth. Jap., 69:1317, 1965.
3. Gifford, S.R., and Marquardt, G.: Arch. Ophth., 21:211, 1939.
4. Hasegawa, S.: Acta Soc. Ophth. Jap., 36:66, 1932.
5. Hornicker, D.: Arch. f. Ophth., 123:286, 1930.
6. Masuda, T.: Acta Soc. Ophth. Jap., 20:1518, 1916.
7. Novotny, H.R., and Alvis, D.L.: Circulation, 24:82, 1961.
8. Smith, J.L., David. N.J., Hart, L.M., Levenson, D.S. and Tillet, C.W.: Arch. Ophth., 69:51, 1963.

ELECTRON MICROSCOPIC OBSERVATIONS ON CYSTOID DEGENERATION IN THE PERIPHERAL RETINA

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Introduction

Since Blessig first described cystoid spaces in the peripheral retina and Iwanoff[12] considered the condition as edema of the retina, which was a misnomer as it was not true edema, cystoid degeneration in the peripheral retina named "Blessig-Iwanoff's edema" has been frequently reported based on light microscopic observations.[1,5,6,7,9,18,19,21,24] However, little is known of the pathogenesis of the disease. It has repeatedly been discussed about the marked predilection of cystoid degeneration for the periphery and the posterior pole, the nature and the probable sources for production of the contents of the cystoid space and the relation of the disease to alterations of retinal blood vessels.

On the other hand, interest in the structure and physiology of neuroglial cells especially the Müller's fiber (or the Müller's cell) in the retina has been taken by recent electron micro-

scopic[3,4,8,11,23] and histochemical studies,[2,12,15] which suggested that the Müller's fiber had important roles in the metabolism of the retina. However, morphological elucidation regarding relationships of the Müller's fiber to the other elements in the retina is rather difficult because of complexity of their cellular architecture in the normal retina. It is, therefore, necessary to study their alterations in pathological states of the retina in order to further clarify the function of the Müller's fiber.

In the present study, the fine structure of cystoid degeneration in the peripheral retina was examined and the relation of Müller's fibers to neuronal elements was elucidated. One purpose of this paper was to evaluate critically light microscopic contributions of cystoid degeneration. The second purpose of this presentation was to describe the relationship between Müller's fibers and neuronal elements. Some possible etiologic factors of the disease were additionally discussed.

Materials and Methods

For the light microscopy, celloidin-embedded sections obtained from autopsy eyes were available.

Materials for the electron microscopic study were obtained from 9 human eyes which were removed from patients of 17 to 62-year-old with orbital tumor or maxillary cancer. Three of them were found to have cystoid degeneration in the peripheral retina and the others appeared to be normal ophthalmoscopically.

Immediately after enucleation, tissues were fixed in 1 or 2 % phosphate buffered osmic acid,[16] dehydrated through ascending concentration of ethanol and embedded in Epon 812.[14] Sections were cut on a MT-2 Porter-Blum ultramicrotome, stained with lead salts[17,20] and examined with a Hitachi HS-6 electron microscope.

Observations

Müller's fiber in normanl human retina.

The fine structure of the Müller's fiber in the human retina has been reported in detail[11] and will be summarized here.

Müller's fibers traverse all the layers of the retina in a radial direction between the inner and outer limiting membranes. Most of these cells have their nuclei in the inner nuclear layer. From cell bodies, Müller's fibers extend large processes on both sides of their nuclei, dividing into many side expansions running through the retinal layers. On the vitreal end of the retina, Müller's fibers terminate in fan-shaped processes. On the other hand, the scleral end of the cell is split into many villiform extensions beyond the outer limiting membrane. Villiform extensions of Müller's fibers protrude into the ventricle lumen along the side of the basal half

of the inner segment of the receptor cell, forming "fiber baskets". Each Müller's fiber contains a great number of glycogen granules and smooth-surfaced endoplasmic reticulum throughout its cytoplasm.

In Fig. 1, Müller's fibers are easily distinguished from neuronal elements by the characteristic cytoplasm. Complicated processes of Müller's fibers occupy the entire interspace between neuronal elements.

Light microscopic observations on cystoid degeneration in the peripheral retina.

Cystoid degeneration is to be frequently found in the peripheral retina by the light microscope (Fig. 2). Cystoid spaces often appear to begin in the inner nuclear and the outer plexiform layers. The disappearance of retinal elements may lead to formation of cystoid spaces of various sizes in the middle layer of the retina. The septum between adjacent cystoid spaces seems to be pillars of proliferated glial tissues. As the cyst enlarges, the inner and outer layers of the retina are encroached, so that eventually the retina is separated into two thin sheets, termed "retinoschisis".

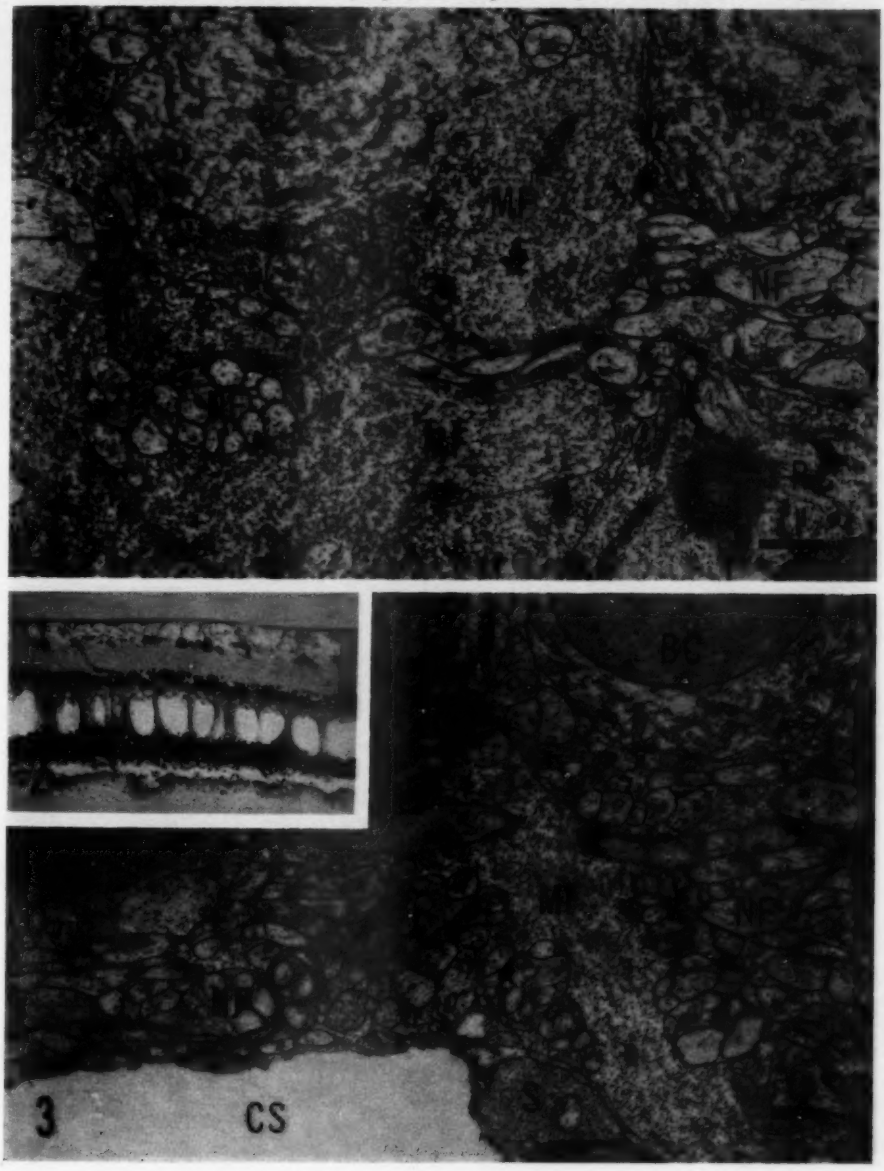
Electron microscopic observations on cystoid degeneration in the peripheral retina.

Large cystoid spaces are formed in the outer plexiform layer in the peripheral retina (Figs. 3, 4, 5, 6, 7, 8, 9 and 10). The inner layer of the retina protrudes into the vitreous lumen and the septum adjacent cystoid spaces is always very thin and is stretched as if the large cystoid space appeared to develop from dilatation of small cystoid space. The contents of the cystoid space are homogeneous and lack any osmiophilic components. The fine structure of retinal elements in the inner layer is retained normally. Neuronal elements show good preservation of synaptic vesicles, synaptic ribbons, neurofilaments and inner and outer segments of receptor cells. The Müller's fiber contains a great number of glycogen granules, smooth-surfaced endoplasmic reticulum, a few mitochondria, fine fibrils, small vesicles and rarely the Golgi apparatus in its cytoplasm. In some sections, however, Müller's fibers in the septum adjacent cystoid spaces include electron dense bodies (Figs. 7, 8 and 9). When multiple cystoid spaces occur in the retina, cystoid spaces communicate with each other in a manner similar to sponge cavities. In a tangential section through cystoid spaces, the septum adjacent cystoid spaces appears to form a network. The septum consists of Müller's fibers and processes of receptor

Key to abbreviations in figures.

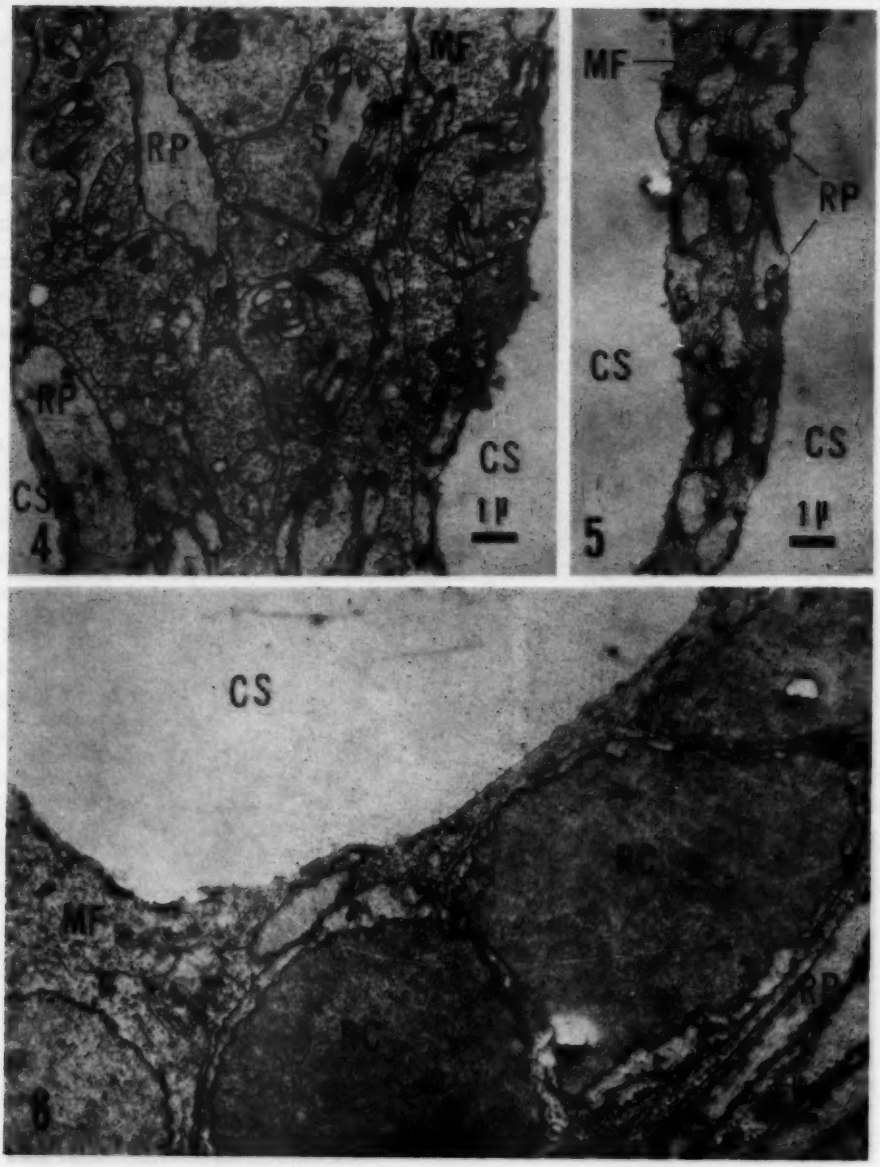
BC : Bipolar Cell
BM: Basement Membrane
CE : Ciliary Epithelium
CS : Cystoid Space
G : Golgi apparatus
IB : Inclusion Body
M : Mesaxon
MF : Müller's Fiber
NF : Nerve Fiber
RC : Receptor Cell
RP : Process of Receptor Cell
S : Synapse
VS : Vitreous Space
ZF : Zonular Fibril

Fig. 1. Outer plexoform layer of the normal retina. MF: Müller's fiber, BC: bipolar cell, RC: receptor cell, NF: nerve fiber. ×10,000.
Fig. 2. Light micrograph of cystoid degeneration in the peripheral retina. ×200.
Fig. 3. Vertical section of the upper wall of cystoid space. The wall of cystoid space (CS) is composed of processes of Müller's fibers (MF) which intervene between neuronal elements. S: synapse, BC: bipolar cell, NF: nerve fiber. ×8,500.

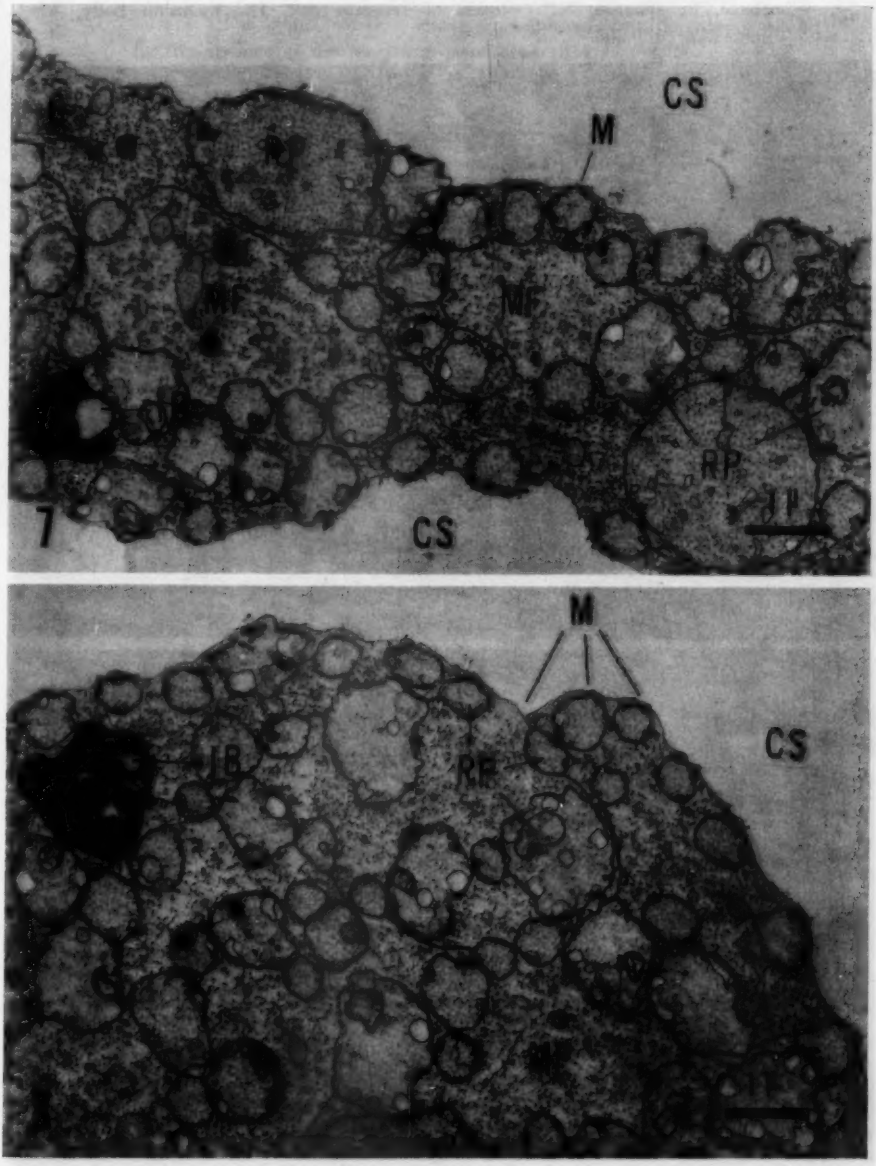


Figs. 4 and 5. Slightly oblique vertical sections of the septa between adjacent cystoid space (CS), which consists of Müller's fibers (MF) and processes of receptor cells (RP). S: synapse. ×7,000.

Fig. 6. Bottom wall of a cystoid space (CS). Receptor cells (RC) are separated from the cystoid space by processes of Müller's fibers (MF). RP:process of receptor cell. ×9,500.



Figs. 7 and 8. Tangential sections of the septa between adjacent cystoid spaces (CS). Processes of receptor cells (RP) are enclosed by Müller's fibers (MF) forming "mesaxons" (M). G: Golgi apparatus, IB: inclusion body. ×13,000.



Figs. 9 and 10. Tangenital sections of the septa. Neuronal elements in the septum preserve their fine structures and the Müller's fiber (MF) contains numerous glycogen granules and smooth-surfaced endoplasmic reticulum. CS: cystoid space, S: synapse, RP: process of receptor cell, IB: inclusion body, M: mesaxon. ×9,000.

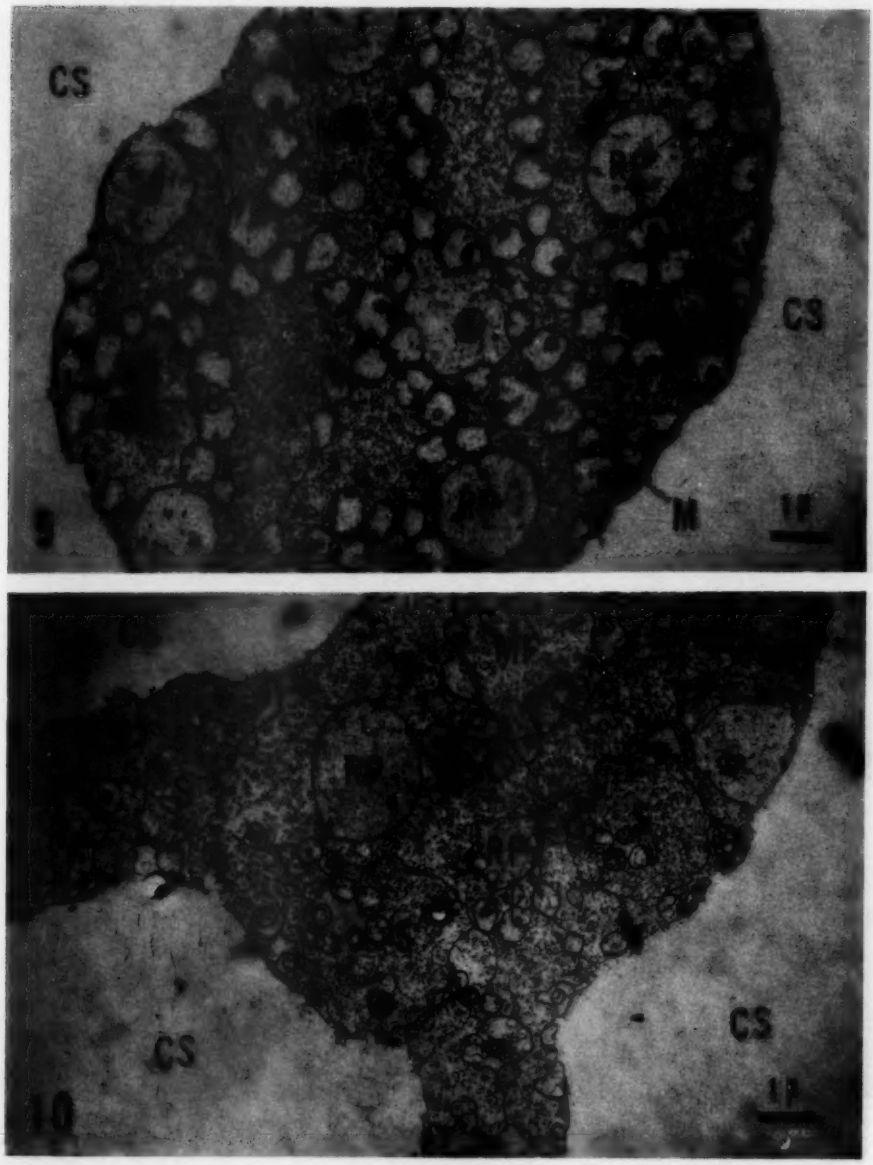
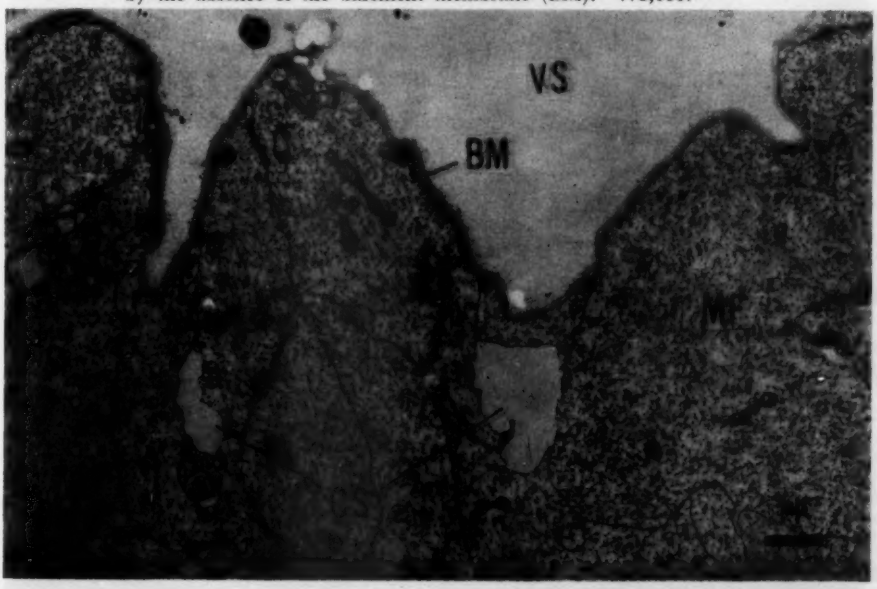
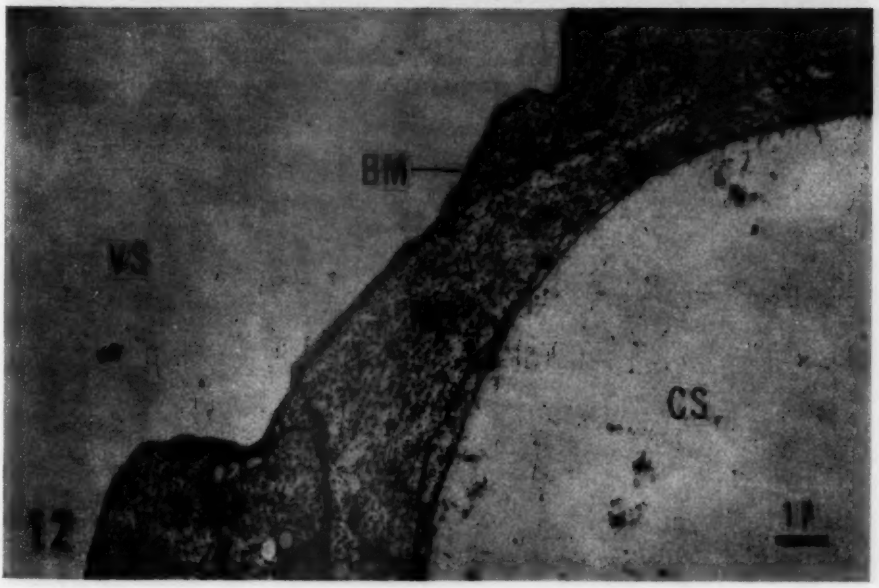


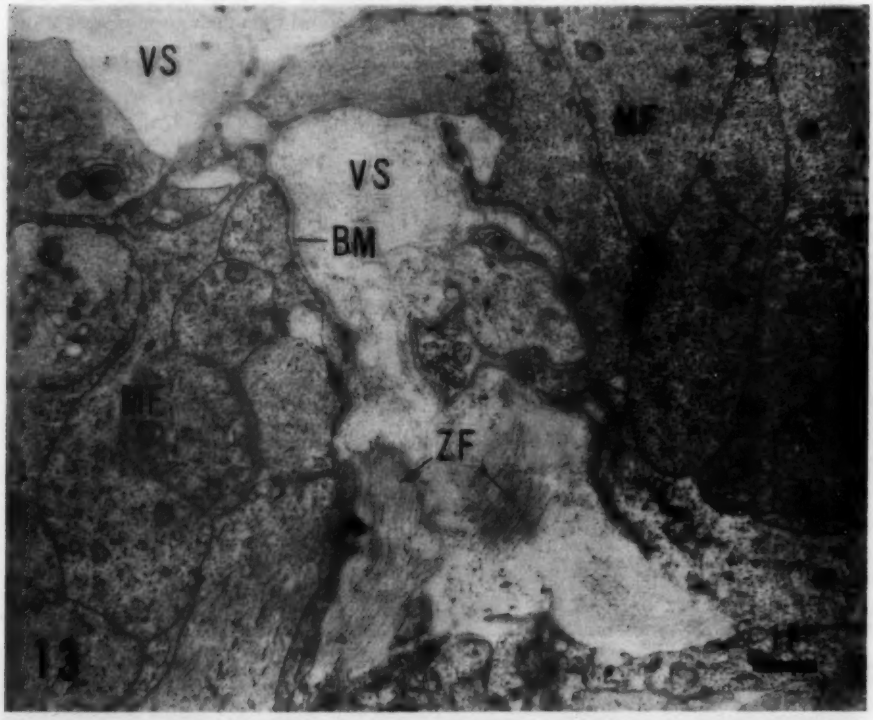
Fig. 11. Inner layer of the peripheral retina near the ora serrata. Microcystoid spaces (CS) are formed in the interspace between adjacent Müller's fibers (MF). VS: vitreous space. BM: basement membrane. ×9,000.

Fig. 12. Portion of a large cystoid space (CS) formed in the inner layer of the ora serrata. The cystoid space is easily distinguished from the vitreous space (VS) by the absence of the basement membrane (BM). ×8,000.





Figs. 13 and 14. Sections of the ora serrata (Fig. 13: vertical and Fig. 14: slightly oblique tangenital section). Zonular fibrils (ZF) extend into the deep crypt of the retina. VS: vitreous space, MF: Müller's fiber, BM: basement membrane, CE: ciliary epithelium. ×10,000.



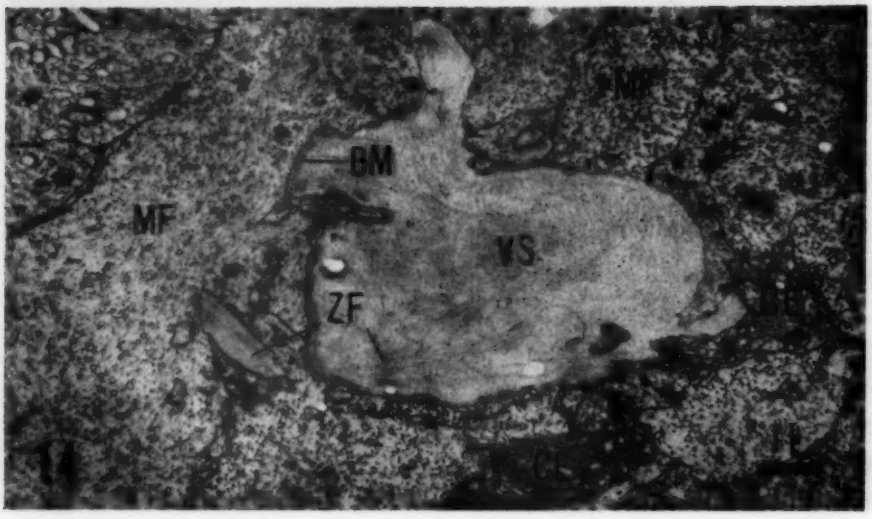
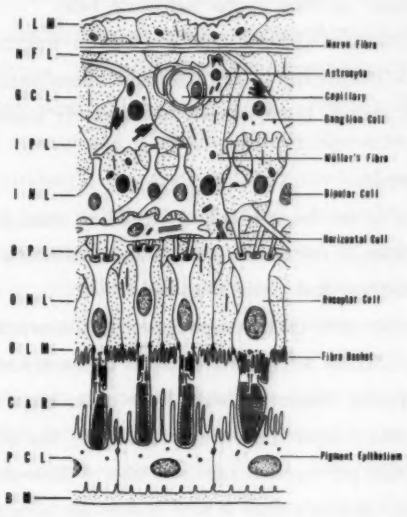


Fig. 15. Schematic drawing of the cellular architecture in the normal retina. ILM: inner limiting membrane, NFL: nerve fiber layer, GCL: ganglion cell layer, IPL: inner plexiform layer, INL: inner nuclear layer, OPL: outer plexiform layer, ONL: outer nuclear layer, OLM: outer limiting membrane, C & R: cone and rod, PCL: pigment cell layer, BM: Bruch's membrane.

Fig. 16. Schematic drawing of cystoid degeneration in the peripheral retina. Large cystoid spaces are formed in the outer plexiform layer.



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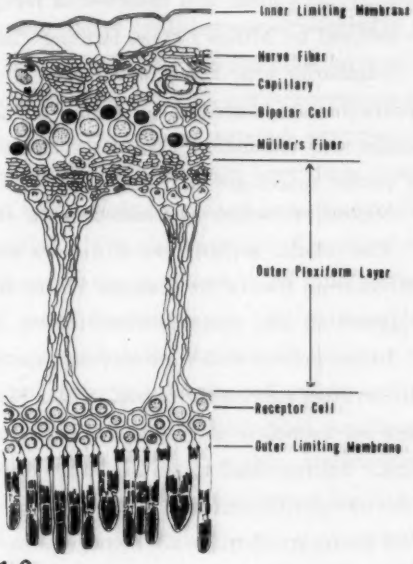

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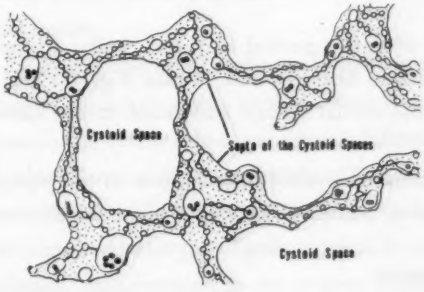
Fig. 17. Schematic drawing of a tangenital section through the septa in cystoid degeneration. The septum between adjacent cystoid spaces consists of Müller's fibers and processes of receptor cells.

Fig. 18. Schematic drawing of the relationship between Müller's fibers and processes of receptor cells. Processes of receptor cells are enclosed by Müller's fiber forming "mesaxons".



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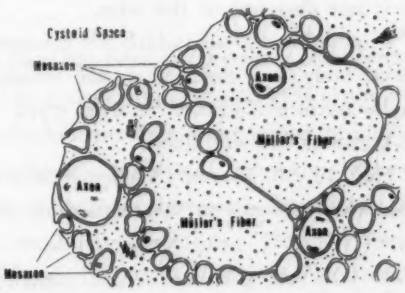

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cells (Figs. 3, 4, 5 and 6). The wall of cystoid space is composed of attenuated processes of Müller's fibers, which intervene between neuronal elements in the septum. Sometimes a part of neuronal elements face the cystoid space, but naked neuronal elements free from covering of tongue-like processes of the Müller's fiber were never observed in the cystoid space. Tangentially sectioned at the level of the septum (Figs. 7, 8, 9 and 10), an intimate relationship between Müller's fibers and processes of receptor cells is observed. Processes of receptor cells are enclosed by Müller's fibers forming "mesaxons" as seen in the Schwann cell.

Capillaries near the cystoid space show the hyalinization which electron microscopic appearance was described by Ikui et al[10] as the thickening in the basement membrane with vacuoles and reticular fibrils. In the posterior retina, blood vessels are severely hyalinized but cystoid spaces are not detectable.

Electron microscopic observations on the ora serrata.

The cellular architecture of the ora serrata of the human retina, which is considerably different from that of the posterior retina, with special reference to relations of Müller's fibers to pigmented and non-pigmented ciliary epithelium has been reported.[22]

In the present study, microcystoid spaces occur with greater frequency on the ora serrata than the other parts of the retina. Figs. 11 and 12 show microcystoid spaces in the ora serrata, which are formed in the interspace between adjacent Müller's fibers of the inner layer of the retina. Microcystoid spaces are easily distinguished from the vitreous space by the absence of the basement membrane at the boundary of the surrounded Müller's fiber. The contents of the microcystoid space are homogeneous. The Müller's fiber is well preserved, even in the region of somewhat larger cystoid space (Fig. 12) the Müller's fiber is distributed by numerous glycogen granules and smooth-surfaced endoplasmic reticulum throughout its stretched cytoplasm.

Capillaries of 17-year-old female retina with microcystoid formation in the ora serrata appear to change variably from the posterior retina to the ora serrata. Capillaries near the ora serrata are more severely hyalinized than those in the posterior retina, where cystoid formation is not observed in this case.

In addition, zonule, which are composed of closely packed filaments similar to those of vitreous body, are observed in the retinal surface near the ora serrata (Figs. 13 and 14). Zonular fibrils extend into the deep crypt in the retinal surface and attach to the basement membrane of Müller's fibers.

Figs. 15, 16, 17 and 18 schematically summarize cystoid degeneration in the peripheral retina with reference to the relationship between Müller's fibers and neuronal elements.

Discussion

The pathology of cystoid degeneration based on light microscopy was described as follows[9].

Peripheral cystoid degeneration first appears immediately behind the dentate processes of the retina at the ora serrata as small isolated cystoid areas. They increase in size both by accumulation of mucoid materials and by coalescence with adjacent cysts. These cysts arise first in the outer plexiform layer, usually encroaching upon the inner and outer layers. Coalescence produces stretching of Müller's fibers. Eventually the cysts extend almost to the inner and outer limiting membranes, and their walls are formed of compressed glial fibers.

In the present observation using electron microscope, large cystoid spaces are formed in the outer plexiform layer in the peripheral retina, and microcystoid spaces in the inner layer of the ora serrata. The contents of the cystoid spaces, whether large or small, are homogeneous and lack any osmiophilic components. Neither fibrin fibrils nor mucoid materials were revealed in the cystoid space. Remains of broken-down neuronal or glial elements were never observed. Although the nature of the contents of cystoid space might be a key to pathogenesis of the disease, the character of cyst fluid has not been studied. The ultrastructure of neuronal and glial elements remained in the region of cystoid degeneration appeared to be quite normal. Synaptic complex was preserved in the stretched outer plexiform layer. No mitochondrial swelling was found in the inner segments, no deformity of lamellar structure in the outer segments of receptor cells. The Müller's fiber contained glycogen granules and smooth-surfaced endoplasmic reticulum throughout its cytoplasm. Although the Müller's fiber occasionally included dense bodies in its cytoplasm, it was obscure whether they were the manifestation of degenerating process of the fiber. The septum between adjacent cystoid spaces consisted of Müller's fibers and processes of receptor cells and the wall of cystoid space was composed of attenuated processes of Müller's fibers. There was no evidence in the present cases that pillars of adjacent cystoid spaces were composed of proliferated glial tissues as previously described. Tangential sections clearly showed the relationship between Müller's fibers and processes of receptor cells. Processes of receptor cells near the surface of the cystoid space were covered by tongue-like processes of Müller's fibers, forming "mesaxons" as seen in the Schwann cell. Naked processes of receptor cells without covering of Müller's fibers were never observed in the cystoid space.

As already discussed in the previous report,[11] there has been no agreement concerning the embryological origin of the Müller's fiber.[3,4,8,23] In comparing neuroglial cells in the retina to those in the brain, it has been obscure whether the Müller's fiber is a modified form of some glial cells in the brain or essentially different from those in the brain. According to present observation, it might be considered that the Müller's fiber corresponded to the Schwann cell in the peripheral and the oligodendroglia in the central nervous systems. The morphological correlation, demonstrated in the present study, between the Müller's fiber and neuronal elements would permit the postulation that the Müller's fiber had important roles in supporting

and nutrition of neuronal elements in the retina.

Etiologic factors in cystoid degeneration that have been advanced in the past were as follows:

1) Age (Henle[7] Iwanoff[12])
2) Primary shrinkage of cells (Ginsberg[6])
3) Artifact (Pau[19])
4) Disturbance of circulation (Iwanoff,[12] Bruno,[1] Vogt[24])
5) Poor nutrition due to vascularization (François and Rabaey[5])
6) Motility of accommodation (Teng and Katzin[21])

Age was definitely an important factor in cystoid degeneration. The condition might be observed by the light microscope in eyes of almost all patients of far advanced age. Cystoid spaces, however, were observed likewise in the periphery of the retina of infants, and even in eyes of newborn babies (Ochi,[18] Teng and Katzin[21]). In the present observation by the electron microscope, microcystoid spaces were frequently found at the ora serrata of 17-year-old female retina.

The primary shrinkage of the retinal cells and the artificial change produced during preparation were not favorable for the pathogenesis of cystoid degeneration from the evidence that the fine structure of retinal cells remained unchanged in the region of cystoid degeneration.

Attention has been drawn previously to the vascular change for the pathogenesis of cystoid degeneration. Iwanoff[12] associated the condition in senile retina with atheromatous change of retinal blood vessels and attributed the development of degeneration to edema of the retina through damage to the capillary. Bruno[1] also thought it might be due to a change in permeability of the capillary wall. Vogt[24] demonstrated ophthalmoscopically the close relationship between obliteration of retinal blood vessels and cystoid degeneration of the retina. By the electron microscopy, capillaries near the cystoid spaces revealed the thickening in the basement membrane with apperance of vacuoles and reticular fibrils. Ikui et al[10] regarded the thickening in the basement membrane with vacuoles and reticular fibrils to be identical condition with hyalinization described by the light micorscopy. Moreover they pointed out the blood vessel in the peripheral retina as one of predisposed portion to sclerotic changes. Their concept was confirmed in the case of 17-year-old female by the fact that capillaries near the ora serrata were more severely hyalinized than those in the posterior retina. However, the development of cystoid degeneration could not always be attributed only to changes of blood vessels, because of low incidence of cystoid formation in the posterior retina, except in the macula there being avascular area, in spite of the sclerotic change of blood vessels.

François and Rabaey[5] described that cystoid degeneration usually had a marked predilection for two areas: the periphery and the posterior pole, where the retina was not only thin-

nest but also relatively the least vascularized. In the present study, microcystoid spaces were frequently found at the ora serrata without cystoid formation at any other parts of the retina. The outer plexiform layer, where cystoid spaces usually began, was considered to be the farest region from the capillary distribution of the retina and the choroid. And the peripheral retina, where only a single layered wide-meshed network of capillaries was distributed with avascular areas at the extreme margin, would be more readily affected by various factors.

Teng and Katzin[21] considered the motility of accommodation as an important and causative factor in cystoid degeneration. According to their postulation, a spongy structure formed at the ora serrata of the retina would be desirable physiologically, which worked in order to strengthen the peripheral parts of the retina and cushion the forces during accommodation.

As already discussed, the cellular architecture of the peripheral retina was considerably different from that of the remainder. Therefore, it is reasonable to regard the peripheral retina near the ora serrata as predisposed portion to cystoid formation. In addition, zonular fibrils were observed in the retinal surface of the ora serrata in the course of this study. Closely packed filaments were identified as a part of the orbiculo-posterior capsular fibers originated from the ora serrata, fibrils extending into the deep crypt in the retinal surface and attaching to the basement membrane of Müller's fibers of the ora serrata. It might be postulated that the zonular fibrils and the meridional fibers of the ciliary muscle created two opposing forces at the ora serrata during accommodation.

From the results obtained by the electron microscopic observation, it might be concluded that some possible etiologic factors co-operated to produce cystoid degeneration in the peripheral retina.

Summary

Cystoid degeneration in the peripheral retina was examined with the electron microscope.

Large cystoid spaces are formed in the outer plexiform layer in the peripheral retina. Near the ora serrata, microcystoid spaces are frequently formed in the interspace between adjacent Müller's fibers. The contents of cystoid spaces are homogeneous and lack any osmiophilic components. The wall of the cystoid space is composed of attenuated processes of Müller's fibers. The septum between adjacent cystoid spaces consists of Müller's fibers and processes of receptor cells. Naked processes of neuronal elements are not seen in the cystoid space. An intimate relationship between Müller's fibers and processes of receptor cells is observed, forming "mesaxons" as seen in the Schwann cell. The fine structure of residual elements in the region of cystoid degeneration is normal. Capillaries near the cystoid space are sclerotic with vacuoles and reticular fibrils in the thickened basement membrane. In the peripheral retina near the ora serrata, zonular fibrils extend into the deep crypt in the retinal

surface and attach to the basement membrane of Müller's fibers.

Some possible etiologic factors of cystoid degeneration were additionally discussed.

REFERENCES

1. Bruno, G.: Arch. f. Augenh., 110:183, 1937.
2. Cogan, D.G., and Kuwabara, T.: J. Histochem. Cytochem., 7:334, 1959.
3. Cohen, A.I.: J. Anat. Lond., 108:179, 1961.
4. Fine, B.S., and Zimmerman, L.E.: Invest. Ophth., 1:304, 1962.
5. François, J., and Rabaey, M.: Brit. J. Ophth., 37:601, 1953.
6. Ginsberg, S.: Grundriss der Pathologischen Histologie des Auges, Berlin, S. Karger, 1903, p. 323
7. Henle, J.: Arch. Ophthl. Graefe's, 156:59, 1955.
8. Hogan, M.J., and Feeney, L.: J. Ultrastruct. Research, 9:47, 1963.
9. Hogan, M.J., and Zimmerman, L.E.: Ophthalmic Pathology, Philadelphia, Saunders Co., 1962, p. 550.
10. Ikui, H., Mimatsu, T., Sugi, K., and Tominaga, Y.: Jap. J. Ophth., 8:35, 1964.
11. Inomata, H.: Acta Soc. Ophth. Jap., 69:2133, 1965.
12. Iwanoff, A.: Arch. f. Ophth., 15:88, 1869.
13. Kuwabara,T., and Cogan, D.G.: J. Histochem. Cytochem., 7:327, 1959.
14. Luft, J.H.: J. Biophys. Biochem. Cytol., 4:475, 1958.
15. Marchesi, V.T., Sears, L.M., and Barrhett, R.J.: Invest. Ophth., 3:1, 1964.
16. Millonig, G.: J. Appl. Physiol., 32:1637. 1961.
17. Millonig, G.: J. Biophys. Biochem. Cytol., 11: 736, 1961.
18. Ochi, S.: Am. J. Ophth., 10:161, 1927.
19. Pau, H.: Arch. Ophth. Graefe's, 158:558, 1957.
20. Reynolds, E.A.: J. Cell Biol., 17:208, 1963.
21. Teng, C.C., and Katzin, H.H.: Am. J. Ophth., 36:29, 1953.
22. Ueno, K., and Inomata, H.: Fol. Ophth. Jap., 16:761, 1965. (in Japanese)
23. Villegas, G.H.: J. Gen. Physiol., 43 (Suppl. 2):15, 1960.
24. Vogt, A.: Disease of the retina, Philadelphia, Blakiston Co., 1947, p. 469.

ELECTRON MICROSCOPIC OBSERVATION OF HUMAN OPTIC NERVES

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In 1959 Yuri had reported his electron microscopic investigation on the optic nerve of the normal mouse. In 1961 and 1962 from our department, Murai and Komai have made electron microscopic studies of the pathological changes of the optic nerves in vitamin A & B_1 deficiencies and also with the case of secondary degeneration. In the present study, the author has tried to observe the ultrastructure of the normal optic nerve of human beings.

Materials and Methods

From the maxillary cancer patients, numbering twenty five, the entire eyeballs with normal optic nerves were excised, and for present observation the frontal part about 8 mm of the optic nerves posterior to the eyeballs were selected. The optic nerves were subdivided in less than 1 mm, and were fixed in 3% $KMnO_4$ solution for 40 minutes. The specimen was placed for 10 minutes each in graded ethanol solution, namely, 50%, 70%, 80%, 90%, 95% and 99% solutions, and twice for 20 minutes in 100% ethanol, then twice for 20 minutes in propylene oxide, and finally for 1 hour in a mixed solution containing equal parts of propylene oxide and epoxy resin. Polymerization embedding was done with epoxy resin in an incubator at 35 °C for 12 hours, at 45 °C for 12 hours, and at 60 °C for 24 hours. An electron microscope of HITACHI HU-11 Type was used for this study.

Results

It has been observed that the fasciculi of nerve fibers are divided into by a system of septa, which are continuous with the pial sheath. The septa is made up of a framework of connective tissue composed of collagenous fibers. Neuroglial framework is forming itself into the nerve bundles.

Nerve fiber.

1) Components of myelinated nerve fiber (Fig. 1).

A. Axoplasm.

The axoplasm is limited by a double layered axon membrane, and the axoplasm contains many axon filaments, endoplasmic reticulum, and mitochondria.

i) Axon filament.

Axon filaments are generally about 100 Å in diameter, and look dotlike in a cross section of the nerve, while in a longitudinal section they are seen running straight, and in places obliquely, along the long axis of the nerve.

ii) Mitochondria.

Mitochondria shows various circular shapes in cross section, and rod shapes in longitudinal section.

iii) Endopalsmic reticulum.

Endoplasmic reticulum is observed in almost the whole sections.

iv) Axon membrane.

The axoplasm is surrounded by the double layered axon membrane, which is 100—200 Å in thickness.

B. Myelin sheath.

The myelin sheath appears as alternating concentric dark and light layers with a characte-

ristic repeating phase of about 140 Å.

2) Thickness of myelinated nerve fiber.

The myelinated fibers are 0.1—2.7μ in diameter. And about 83% of these fibers are less than 1.0μ in diameter. The thickness of the myelin sheath are 0.03—0.27μ, and most of them are less than 0.1μ in thickness.

3) Node of Ranvier.

In the longitudinal section, of the node of Ranvier it was possible to observe myelin loops, which had lined up along the long axis of the nerve. Lamellar structure of the myelin sheaths appear to be turning backward and form separate nodal myelin loops. These nodal myelin loops contain the glial cell cytoplasm and hug the axon tightly in the juxta-terminal myelinated region of the node. Endoplasmic reticulum is always observed in the myelin loops.

4) Non-myelinated fiber (Fig. 2 and Fig. 3).

In the cross sections, the non-myelinated nerve fibers are observed gathering as circular manner around the central part of the optic nerve bundles, which are made up with many myelinated fibers.

Glial cells.

In the optic nerve, morphological differentiation can be made into two types of glial cells, i.e. oligodendrocytes and astrocytes.

1) Oligodendrocytes (Fig. 4).

The nuclei are nearly circular. The cytoplasm appears to be small, containing mitochondria, endoplasmic reticulum and Golgi's apparatus. Oligodendrocytes contain hardly any processes, however, short cell processes have been recognized in a few instanses.

2) Astrocytes (Fig. 5, Fig. 6 and Fig. 7).

Astrocytes have an elliptical nuclei and rich cytoplasm including mitochondria, endoplasmic reticulum and Golgi's apparatus. Astrocytes have many, long processes. An astroglial framework migrate into the nerve bundles. The peripheral part of the nerve bundles are filled up with many astroglial cell processes attached to the septal connective tissue and pial sheaths.

Blood vessels.

Blood vessels are observed only in the connective tissue septa, and none in the nerve bundles. The endothelial cells of capillaries are closely packed each other, and are tightly surrounded by the basement membrane peripherally.

Septal connective tissue and pial sheath.

The fasciculi of nerve fibers are divided into small nerve fiber groups by connective tissues septa, which are continuous with the pial sheath. In these septa and pial sheaths, between the connective tissue cells were found the collagenous fibers.

Liquor-opticus barrier (Fig. 8 and Fig. 9).

The central part of the optic nerve bundles are mostly made up with numerous myelinated fibers, though a few oligodendrocytes and astrocytes are present. Around these myelinated fiber groups, there are non-myelinated fibers. But in the peripheral part, abundant astrocytes and astroglial cell processes could be seen, but myelinated nerve fibers were absent. These processes of glial cells are grouped as a layer usually about 10μ in thickness, but close to the pial sheath, the layer of these processes are extremely thick, about 40—50μ. The septal connective tissues intrude into the nerve bundles as a wedge, while the surface of nerve bundles itself is not smooth. That is to say, the nerve bundles are surrounded by groups of glial cell processes, which project into the connctive tissues. The nerve bundles are tightly surrounded by the basement membrane directly and connective collagenous fibers peripherally.

Discussion

The fixation of nerve tissues for the electron microscopic study is no easy matter. For the present study, a technique of the nerve fiber employing $KMnO_4$ and embedded with epoxy resin has been tried.

A normal human optic nerve is built up with the pial and septal connective tissues which contains blood vessels, including many myelinated fibers, a few non-myelinated fibers, glial cells and their processes. Observation has been made on the correlations of these components, mainly non-myelinated fibers, properties of node of Ranvier, glial cells and their processes to the axon of the optic nerve, and the possible function of the fine structure of Liquor-Opticus Barrier by means of serial sections.

In a cross section, the non-myelinated fibers have the figures similar to that of the glial cell process. It is hardly possible to differentiate the non-myelinated fiber from glial cell processes. In serial cross sections (Fig. 2 and 3), the author was able to differentiate the ciruclar axon of non-myelinated fibers surrounded by variable forms of the glial cell processes. It has been well demonstrated electron microscopically that the human optic nerve is comprised of certain amount of non-myelinated fibers. Each of the non-myelinated fiber has the axon membrane without lamellar sheaths. These nerve fibers have collected themselves in circular arrangement in the central portion of the optic nerve bundles, which are composed of myelinated fibers, and encircled by end-feet of the glial cell processes.

The ultrastructure of the node of Ranvier has recently been reported that, the nodal myelin loops are made up with the distal folding of the myelin sheaths containing glial cell cytoplasm, and these associated glial cells for the myelination are supposed to be the oligodendrocytes.

With the non-myelinated part of node of Ranvier, the axons are directly surrounded by astroglial cell processes.

The author has been able to differentiate two kinds of glial cells, i.e., oligodendrocyte, astrocyte. The author however, has observed functionally two different types of oligodendrocytes, the first type the cells are found in the central part of the optic nerve bundles surrounded with many myelinated fibers. While, the second type of these are seen in the periphery of the optic nerve bundles mixed with many astroglial cell processes. It has been assumed that these second type of oligodendrocytes are associated with the regulation of the function of the astrocytes which are supposed to be related with the Liquor-Opticus Barrier, because the myelinated fibers are absent in the peripheral part of nerve bundles. (Fig. 8 and Fig. 9)

The form of the astroglial cell processes were studied by the serial sections. Fig. 6 and 7 showed that the astrocyte has many long and winding processes. Especially, the peripheral part of nerve bundles are occupied with astrocytes and abundant astroglial cell processes, so the myelinated nerve fibers are situated at a distance from the septa separated by these groups of glial cell processes.

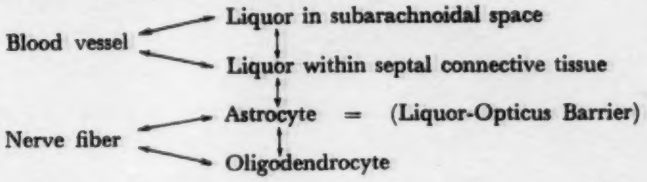
The septa are made up of a framework of connective tissue contiguous with the pial sheath, so that the optic nerve could be divided into many minute nerve bundles by these septa, and these septal connective tissues have intruded into the nerve bundles, the surface of nerve bundles occupy wide area and looks intricated. (Fig. 8 and Fig. 9)

Blood vessels are observed only in the connective tissue septa and pial sheath, but not in the nerve bundles of the normal human optic nerve. This prominent fact arouses an interest concerned in the metabolism of the nerve bundles.

The fact that the fasciculi of human optic nerve fibers are dividable by a system of septa has long been well established. But the ultrastructure of the peripheral part of optic nerve bundles has not been heretofore reported. As has been discussed before, that in the periphery of nerve bundles, glial cell processes are lined up closely so that the myelinated nerve fibers could hardly observed. Further, the surface of nerve bundles are extremely complicated and connective tissue migrated deep into the nerve bundles. This fact seemed to show that the nerve bundles extend the surface like the alveoli of lung tissue to make contact with the collagenous fibers. It is interpreted that the metabolic process of the bundles is dependent upon the liquor as that of blood, and these glial cell processes can serve to transport nutrient substance through blood or liquor to the interior of the nerve bundles which are devoid of blood vessels. The function of astroglial cell processes is, similar to the lymph vessels, possesses an ability of transporting selectively the blood components or cerebrospinal fluid to the nerve fibers. In 1957 Kobayashi and Mukai had proved biochemically, using P^{32}, that the transportation of nutriments to the optic nerve through liquor far exceeds that of through blood

circulation. Present investigation likewise assumes that the fine structure of optic nerve bundles present the possibility of the transporting channel through liquor to the optic nerve through collagenous fibers. Hence, the author wishes to designate this structure as the Liquor-Opticus Barrier from the anatomic point of view. (Fig. 8 and Fig. 9)

The setting could be summarized that:



In view of the function of oligodendrocytes which were found in the peripheral part of the optic nerve bundles, the author has assumed that these oligodendrocytes are related to functional regulation of astrocytes which would serve as an anatomical Liquor-Opticus Barrier. (Fig. 8 and Fig. 9)

Summary

The fine structure of normal optic nerve posterior to the eyeballs of human beings was studied with the electron microscope, applying $KMnO_4$-fixation and epoxy resin polymerization embedding.

The results are summarized as follows.

1) The fasciculi of nerve fibers are divided into by a system of septa, which are continuous with the pial sheath. The septa is made up of a framework of connective tissue composed of collagenous fibers. Between the connective tissue septa, which support the bundles of nerve fibers, a neuroglial framework intrudes itself into the nerve fibers.

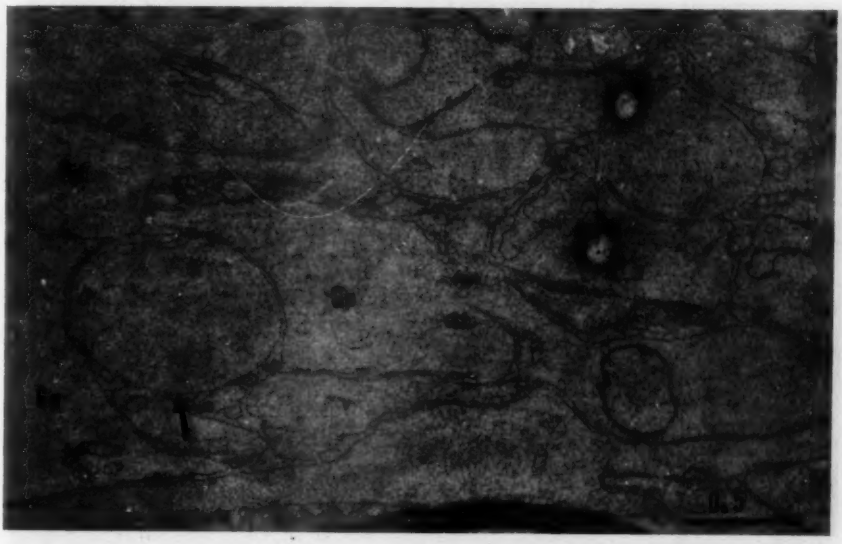
2) The axoplasm of the myelinated fiber contains within itself many axon filaments, endoplasmic reticulum, and mitochondria. With electron microscopy, the myelin sheath

Explanation os abbreviations.

Ast : astrocyte	Gm : glial cell membrane
Axf : axon filament	Gp : glial cell process
Axm : axon membrane	I : internal loop
Bm : basement membrane	M : microbody
Bv : blood vessel	m : mitochondria
Cf : connective tissue cell	MS : myelin sheath
e : endoplasmic reticulum	N : nucleus
G : Golgi's apparatus	Np : nuclear pore
Gc : glial cell cytoplasm	OL : oligodendrocyte

Fig. 1. Cross section of the normal human optic nerve. In the myelinated nerve fiber, the axoplasm is limited by double layered axon membrane. The axoplasm is surrounded by the myelin sheath, which appears compact and shows clearly lamellar structure. The axoplasm contains within itself many dotlike neruofilaments, endoplasmic reticulum, and mitochondria. The spaces between myelinated nerve fibers are filled tightly with glial cell processes. ×60,000

Fig. 2 and Fig. 3 (serial sections). Elliptical shapes of axon of non-myelinated fibers are observed at the arrows in two figures. The forms of the glial cell processes arround the axon (arrow) are very variable in every serial sections. ×40,000



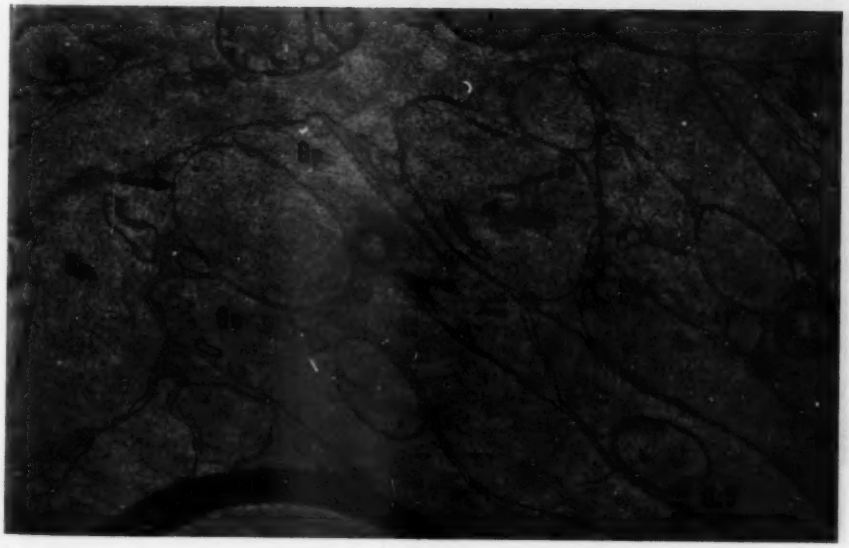


Fig. 4. Oligodendrocyte has little cytoplasm with scanty processes. The cytoplasm contains a moderate quantity of endoplasmic reticulum, a moderate number of mitochondria, and a few groups of Golgi's apparatus. The nuclear membrane appears as a very clear double membrane and has many well defined nuclear pores. The processes of oligodendrocyte are rather short as shown by the arrows. ×20,000

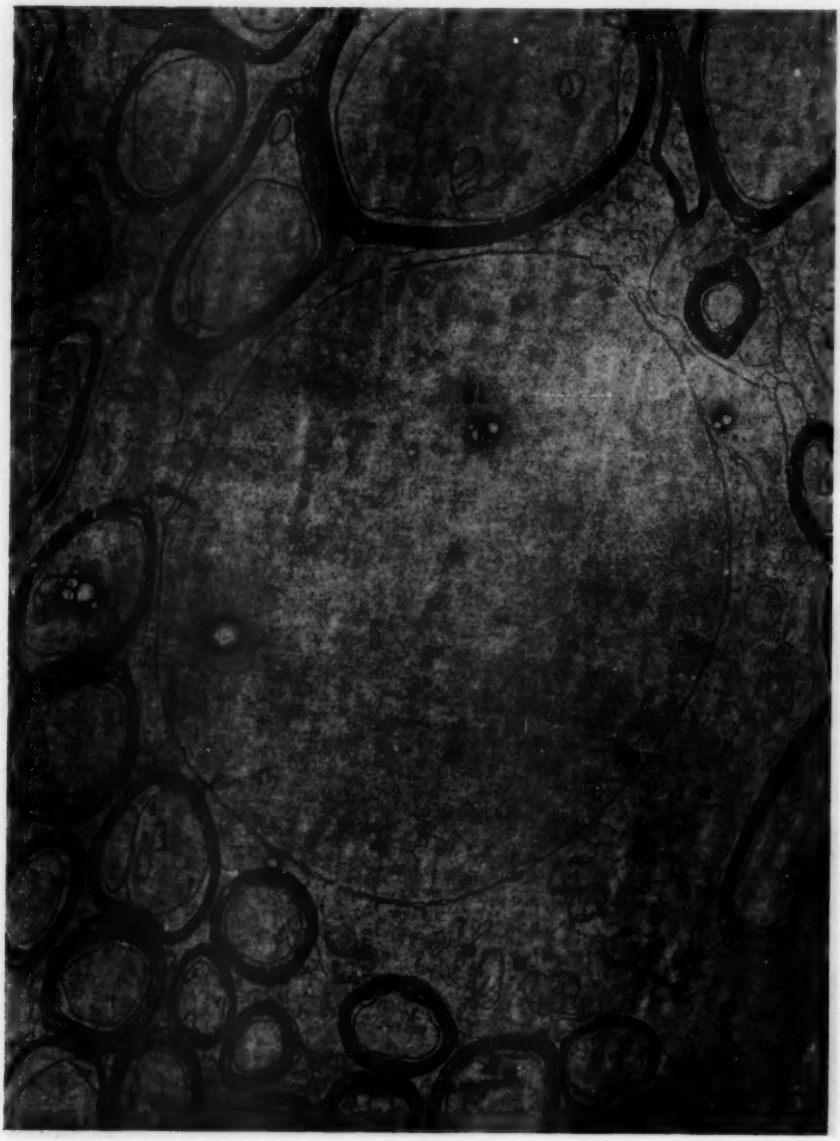


Fig. 5. Astrocyte has rich cytoplasm with many processes. And in the cytoplasm of astrocyte it contains mitochondria, endoplasmic reticulum, Golgi's apparatus and microbodies. The microbody shows a high electron density and is about 0.4μ in diameter. ×19,000

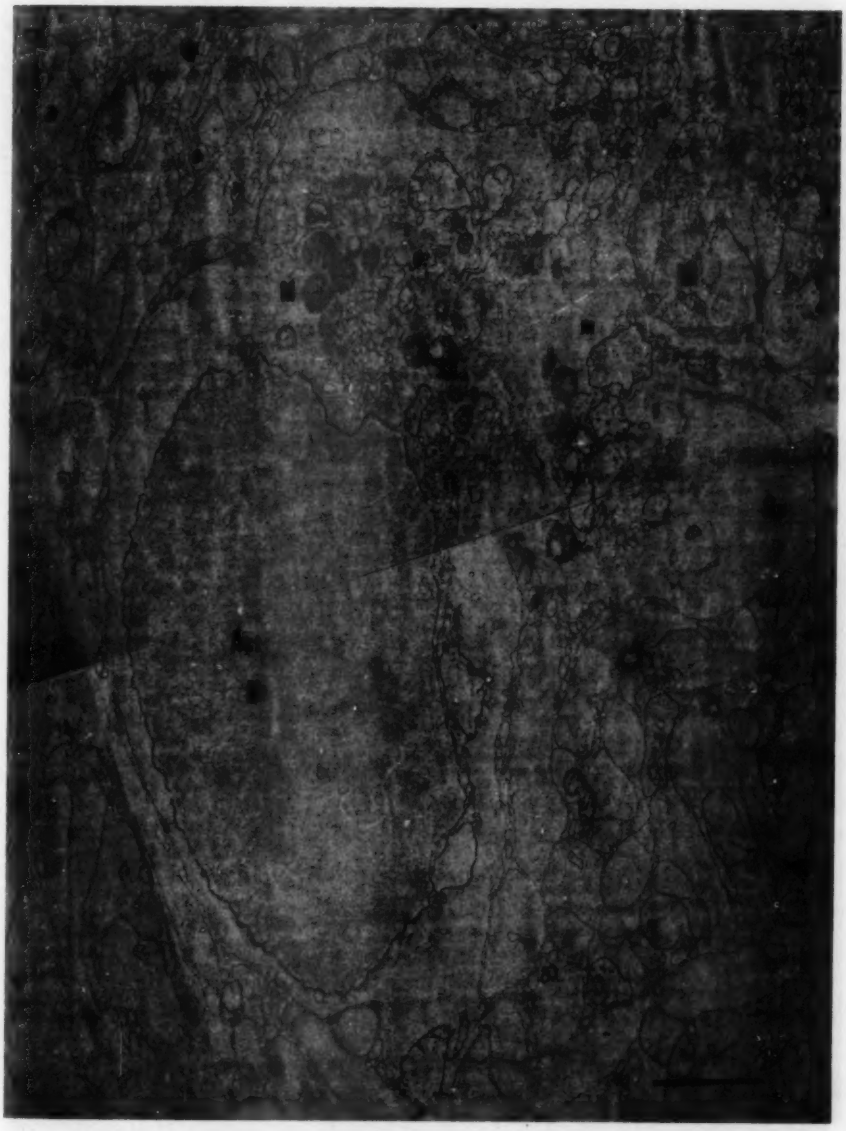


Fig. 6 and Fig. 7 (serial sections). An astroglial cell process in Fig. 6 is interrupted at the arrow, but by the serial observation we can demonstrate this process as continuous as shown in Fig. 7 winding, very long and with changing calibers. ×12,000

Fig. 8. In the middle of this photograph, the connective tissue is projecting into the groups of glial cell processes. The basement membrane is apparently seen between the groups of glial cell processes and the connective tissue. ×18,000

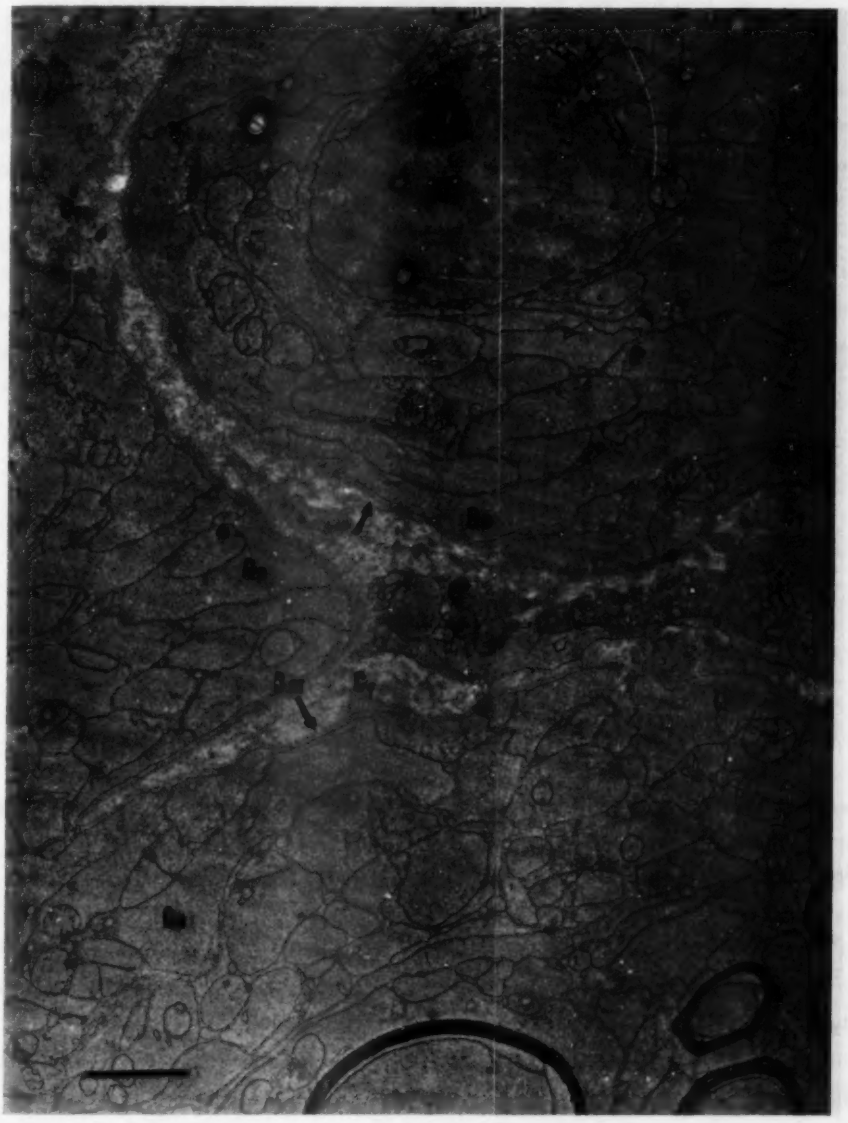
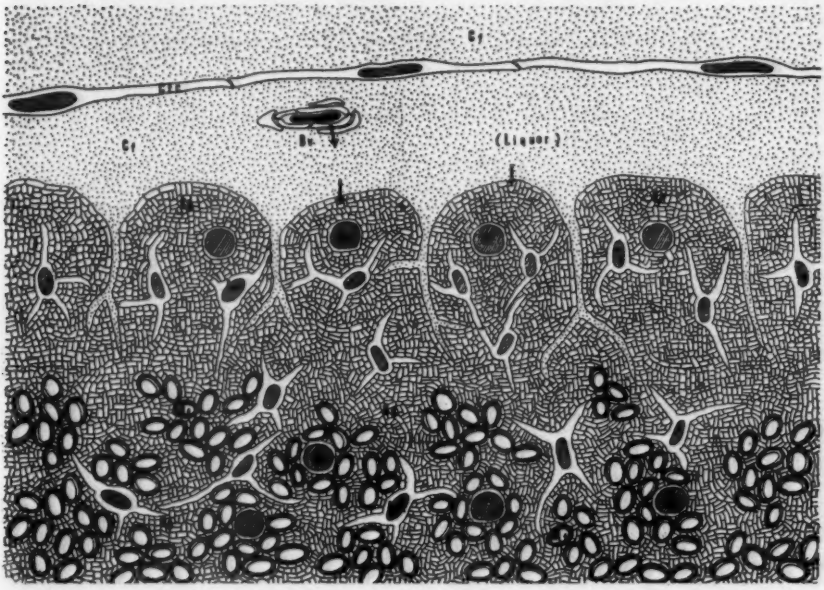


Fig. 9. Diagram of the peripheral part of normal human optic nerve bundle is shown. In the peripheral part of the bundle, astrocytes and astroglial cell processes are abundant, but myelinated fibers are absent. The septal connective tissues are intruding into the nerve bundles as a wedge and the surface of nerve bundle itself is not smooth. Oligodendrocytes are also situated in the astroglial process group. Blood vessels are observed only in the connective tissue septa, but not in the nerve bundle. The author assumes the possible transportation of nutriments to the intracellular tissue of the nerve bundle through the collagenous fiber layers at arrows.



showed alternating concentric dark and light layers with a characteristic repeating phase of about 140Å and their thickness was found 0.03—0.27μ.

3) The myelinated fiber is 0.1—2.7μ in diameter. About 83% are less than 1.0μ in diameter.

4) The myelin sheaths folded individually and form the nodal myelin loops respectively at the node of Ranvier. The nodal myelin loops include glial cell cytoplasm and hug the axon tightly in the juxta-terminal myelinated region of the node.

5) The non-myelinated fibers are observed mostly in the subcircumferential area of nerve bundles. They are surrounded chiefly by end-feet of the astroglial cell processes.

6) Two kinds of glial cells, i.e. oligodendrocyte, astrocyte are observed. In general, it is assumed that the oligodendrocytes are only related to the myelinating process, present study revealed the facts that two types of oligodendrocytes differ in their function. The astro-

cyte has many processes which are extremely elongated and winding, especially, the peripheral part of nerve bundles are filled with these processes, and myelinated nerve fibers are situated at a distance from the septa separated by these groups of glial cell processes.

7) Blood vessels are observed only in the connective tissue septa and the pial sheath, but none found in the nerve bundles.

8) In the peripheral part of nerve bundles, astrocytes and astroglial cell processes are abundant, and myelinated nerve fibers are absent. The septal connective tissues intrude into the nerve bundles as shape of a wedge and the surface of nerve bundles itself is least smooth. This fact indicates that the nerve bundles are extended the surface to make wide contact with the liquor.

9) These astroglial cell processes can serve to transport nutriments from the liquor to the inside of the nerve bundles, which lack blood vessels. Therefore, the fuction of the astroglial cell processes can be regarded as a lymph vessels and the oligodendrocytes make myelinization and also act as a regulator of the function of the astrocyte.

REFERENCES

1. Honjin, R.: Folia Anat. Jap., 29:117, 1956.
2. Kobayashi, A.: Acta Soc. Ophth. Jap., 62:2562, 1958.
3. Komai, T.: Acta Soc. Ophth. Jap., 66:1353, 1962.
4. Maturana, H.R.: J. Biophysic and Biochem. Cytol., 7:107, 1960.
5. Mukai, A.: Acta Soc. Ophth. Jap., 64:1041, 1960.
6. Murai, M.: Acta Soc. Ophth. Jap., 65:1631, 1961
7. Wolter, J.R. und Liss, L.: v. Graefes Arch. Ophthal., 158:1, 1956.
8. Yamamoto, T.: Acta Soc. Ophth. Jap., 69:1527, 1965.
9. Yamamoto, T.: Fol. Ophth. Jap., 16:161, 1965.
10. Yuri, Y.: Acta Soc. Ophth. Jap., 63:2673, 1959.
11. Yuri, Y.: Jap. J. Ophth., 4:48, 1960.

THE OSCILLATORY POTENTIAL IN THE RETINA AND THE LATERAL GENICULATE BODY OF RABBITS

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In a series of studies on the visual pathway of rabbits we have recorded the oscillatory potentials evoked by a light flash from the cornea, the retina and the optic nerve[8]. It may be concluded from the results that all of these oscillations exhibit close time relations. This suggests that rhythmic activity of some elements in the retina has propagating nature, and

if so, it will be expected that oscillatory response of the same or similar frequency can be reproduced in further proximal parts of the visual system.

The present study has been carried out on such problems as follows: (1) Recording of the oscillatory responses, mainly 'on' rhythm, in the lateral geniculate body of rabbit, and detection of a site where marked rhythmic response is arising. (2) Identification of the oscillatory potential in the geniculate by comparison of electrically and photically elicited responses. (3) Observation on tne relation between the geniculate response and corneal electroretingram (ERG) or intraretinal ERG.

Methods

Albino rabbits, weighing 2.0 to 2.5 kg, were anesthetized by intraperitoneal injection of 25% urethane, and mounted on the stereotaxic apparatus. The skin of each head and upper lid were widely resected and a small hole was made on each skull just above the lateral geniculate. The recording electrode was usually advanced downward through this hole into the brain. In some cases, the skulls and the meninges were partly opened and posterior half of the brain was carefully removed, and the electrode was manipulated under the direct vision for the geniculate. Exposed brain surface was always covered with a pool of mineral oil. After the experiments the brain was placed in formalina solution and its serious sections were histologically examined on the location of the electrode tip.

Recording electrodes for the geniculate response were two kinds; one was a steel neelde electrode, 5—10μ in tip diameter, and the other was a silver wire electrode, 100—500μ in tip diameter. The latter was used when the response was compared with the corneal ERG, which was recorded with similar gross electrode. The intraretinal ERG was taken with a microelectrode consisting of steel needle, 1—2μ in tip diameter. This electrode was inserted into the retina from choroidal side at the posterior part of the eye ball where a scleral incision has previously been made. The tip of the electrode was adjusted so as to locate in the middle layer of the retina, from which the negative b-wave was usually recorded as well as positive one when a diffuse light was used as a stimulus. Indifferent electrode made of silver plate was placed on the midline of the head. Signals from these electrodes were amplified by a condensor-coupled amplifier (AVB-1) and displayed on two beams oscilloscope (VC-6).

A photic stimulater, delivering 10μ sec xenon flash (1.4 Joule/flash), was used in all cases. The maximum light was reduced by neutral filters of various densities ranging from 0.5 to 2.5. When the electrical stimulation was needed, a concentric stimulating electrode was inserted into the exposed optic nerve just proximal to the eye ball, delivering a square pulse of usually 0.2 msec and 5—20 Volt.

Most of the experiments were done in a dark room except those on photopic responses. In this paper upward deflections in the records indicate the positivity at the recording electrode. The beams of the oscilloscope were started with the onset of a light flash except in such cases as subjected to an electrical stimulation.

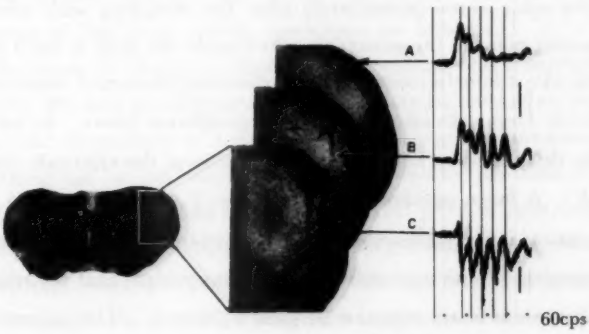
Results

1. Evoked response from various parts of the lateral geniculate body of a rabbit.

Shape of the evoked responses to a flash light showed considerable variations according to the positions of the electrode tip in the geniculate. In rabbit, as well known, optic tract fibers are widely distributed over lateral and superior surface of the geniculate where they penetrate into the geniculate neucleus, forming synaptic junctions with postsynaptic elements. There are no such discernible cell layers as in cats or monkeys, and it seems that most of the postsynaptic fibers converge into ventrorostral part of the geniculate, forming radiation fiber bundle which runs forward for a distance. Some of the tract fibers pass through the posterior part of the geniculate and reach the superior colliculus. From such anatomical circumstances it was expected that characteristics of summed potential evoked in rabbit's geniculate might be recorded from ventrorostral part of the dorsal neucleus. In fact this is what we obtained in our study, and main problems were studied on the responses recorded from this part.

Figure 1 shows different responses as a recording electrode was advanced downwards from the superior surface at the relatively anterior part of the geniculate. When it was located in

Fig. 1. Evoked responses recorded from different parts of the lateral geniculate body. A: the layer of the optic tract fiber, at the depth of 0.2 mm from the superior surface. B: the dorsal neucleus (ND) of the lateral geniculate, at the depth of 2.0 mm. C: lower margin of the dorsal neucleus. Note the difference in calibrations for upper two beams and for the lowest one.
Flash: 1 cps. Calibration: 200μV. Positivity is up in all records.



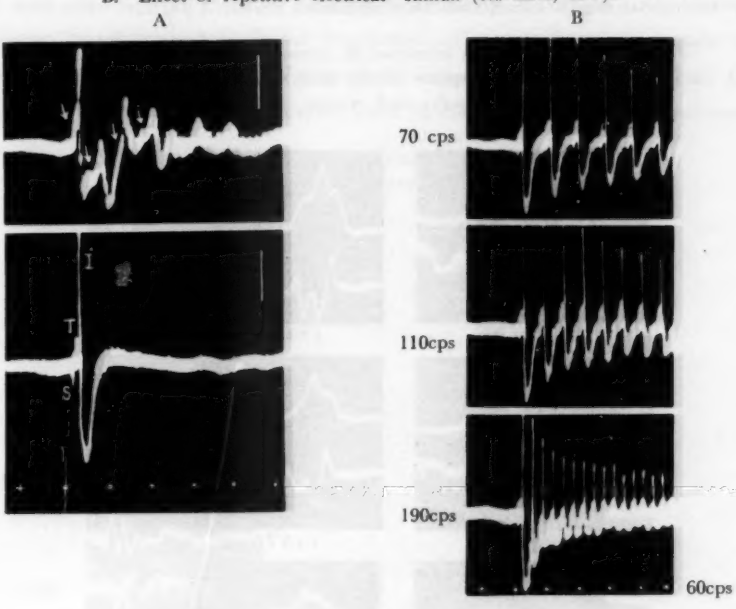
the tract fiber layer (A), a photically evoked response exhibited the same pattern as recorded from the optic nerve, indicating that the response is purely presynaptic. When the electrode was penetrated into a dorsal neucleus (ND) the oscillation became augmented (B) by additional summation with the postsynaptic potential. Close to the lower margin of the dorsal neucleus, the response was consisted mainly of negative deflections on which several impulses were seen, and developed to maximum in size (C) in all recordings. The findings indicate that the recording C is a representative postsynaptic response in the geniculate, particularly of the oscillatory potential. All of the recordings in our study were similar to the latter in shape, although the initial positive wave showed considerable difference in size according to the electrode locations. It appears that in our recordings negative going deflections indicate the postsynaptic actions, whereas positive ones are either pre- or postsynaptic and occasionally the sum of them. Therefore, measurements were made on the periods between negative peaks when if necessary.

It was noticed that the course of the oscillation, ranging from 6 to 10 msec, were hardly changed throughout the electrode penetration as shown by vertical lines in Fig. 1, although minimal delay in time course reasonably occurred in the lowest recording (C). This is an important finding in realizing that the rhythm in the presynaptic volley is faithfully followed by some postsynaptic neurons.

2. Comparison of electrically and photically evoked responses.

From the above described results it appears that the rhythmic action recorded from a certain part within the lateral geniculate is the postsynaptic response to incoming repetitive afferents caused by retinal driving. On the other hand, it is shown that a single electrical stimulation to the optic nerve or tract can elicit repetitive firing of some geniculate neurons.[8] Such a rhythmicity may be a general trend in evoked response induced in the central neurons. Therefore, for identification of photically evoked oscillation in the geniculate it will be needed to compare the results obtained by both electric and photic stimulations. Figure 2 shows a case examined for this purpose. A single shock of 0.2 msec square pulse was applied to the distal end of the optic nerve immediately after the recording with photic stimuli. Accordingly, the recording point in the geniculate was exactly the same in both procedures. The recording by steel needle microelectrode (2μ in tip diameter) showed a response of one or only a few elements, probably large cells associated with large afferent fibers. In such a case polarity of the response may depend upon neumerous factors around the electrode tip and the kind of neurons concerned.[1] A large positive spike (I in Fig. 2 A) was identified as an activity of post synaptic elements as well as following negative wave by the finding illustrated in Fig. 2 B where repetitive electrical stimuli were delivered. It was evident that the response was readily diminished in its size even at lower stimulus frequency (70 cps). This cannot be demonstrable

Fig. 2 A. Comparison of photically (upper beam) and electrically (lower beam) evoked responses. Arrows: presynaptic afferents. S: artefact by a electrid stilulus. T: presynaptic afferent. I: postsynaptic response. Claibration : 200μV.
B. Effect of repetitive electrical stimuli (0.2 msec, 20V).



in optic tract response and is the characteristic of postsynaptic impulse. An afferent volley appeared on the beam as only a small positive hump (T in Fig. 2 A) immediately following a stimulus artefact (S). Postsynaptic response is therefore revealed here as a diphasic potential change in positivenegative sequences without any succession of rhythms. If we suppose this as unitary response to a single afferent, it will clearly be understood that it is repeatedly induced by a series of afferents when a light flash is given (upper beam in Fig. 2 A). This was confirmed by further evidence that each impulse was preceded by a small hump (indicated by arrows in Fig. 2 A) which represents an afferent.

When the frequency of electrical stimuli was increased, the postsynaptic response was largely reduced (Fig. 2B) and yet still maintained considerable level of excitability even at 190 cps. This means that in response to a photic stimulus the geniculate neurons, if not all, can safely follow incoming 'on' or 'off' rhythms which are usually less than 200 cps.

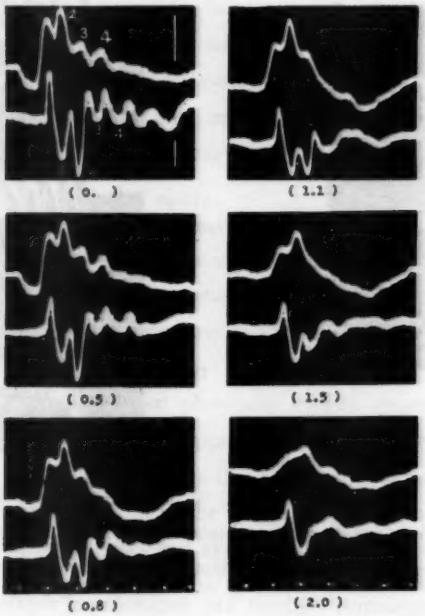
In rabbit's geniculate, Arden and Liu[1] found several types of cells responding to a flash with different behaviors. There should be many other neurons which are unable to respond with such rhythmic manner as presented here. In other experiments we could also record repetitive firing to a single electric shock from such neurons as described by Bishop[2] on cat's

geniculate. However, their periods of 2 or 3 msec were quite different from those under observation.

3. Combined observations on the geniculate response and corneal or intraretinal ERG.

Under various stimulus conditions both responses revealed parallel behaviors. Figure 3

Fig. 3. Effect of stimulus intensities on corneal ERG (upper beam) and the geniculate response (lower beam). Densities of neutral filters are shown under each record. Flash: 1 cps. Calibration: 200μV.

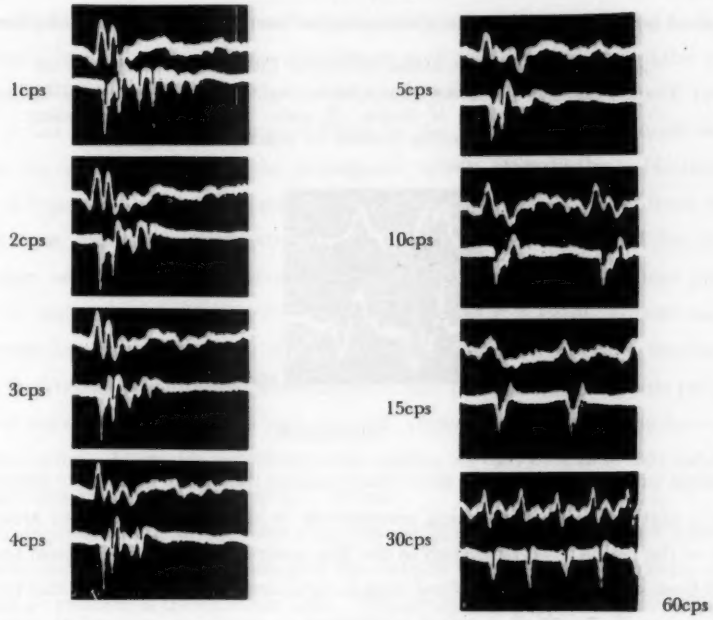


shows simultaneous recordings of corneal ERG (upper beams) and lateral geniculate response (lower beams) to a light flash. The maximum light (density:0) was reduced in intensity by neutral filters of densities from 0.5 to 2.0. The geniculate response was recorded with a gross silver wire electrode which enabled to obtain summed potential from the area as wide as possible, whereas no impulse activities was picked up. Yonemura[10] reported that the periods or frequency of the oscillatory potential on the b-wave could hardly be altered by changing stimulus intensity. This was equally confirmed on the rhythms in the geniculate response. When the measurements were made on the periods between positive and negative peaks indicated by numbers in the figure, their values between corresponding peaks showed close approximation for all recordings. With decreasing stimulus strength the rhythmic actions in either beams were gradually attenuated from later ones. The period between peak 1 and peak 2 was the shortest and that between peak 3 and peak 4 was the longest in both responses. This may be a feature

in an evoked rhythmic response at any part of the neural tissue, but may provide in part an additional support for our arguments.

The effect of flickering light from 1 to 30 cps on the geniculate response was always compared with the intraretinal ERG, because of difficulty of corneal ERG in revealing distinct oscillation to relatively high flicker frequency. The recordings were made with microelectrodes consisting of steel needle (2—5μ in tip diameter). The intraretinal ERG(upper beams in Fig. 4) was taken from the middle layer of the retina with short time constant (0.01

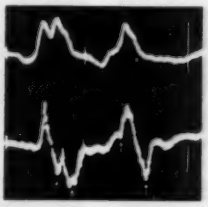
Fig. 4. Effect of flicker on intraretinal ERG (upper beam) and the geniculate response (lower beam). Calibration: 200μV.



sec) by which negative b-wave was eliminated from the records, while the fast components were well displayed. In contrast to Fig. 3, the geniculate response (lower beams) was characterized by marked impulse activity seen on negative deflections. Of these negative wavelets, the first two or three seemed to be purely, 'on' response and the following ones be 'off' rhythms. The latters could be recorded from the optic nerve as well, but quite weak in the retina under ordinary experimental conditions, probably because of minority in concerned members. In both responses, main deflections showed close relations in time course and the periods between the first two peaks were equally shortened very slightly at more than 4 cps. As the flicker frequency was increased the wavelets were gradually decreased in their numbers and finally, more than 15 cps, their shapes became almost monophasic. Delivering

paired flashes similar parallel behaviors in both responses could be seen as shown in Fig. 5 where the experiment was done under the photopic condition (500 lux). Responding to the first flash there appeared two distinct wavelets in the corneal ERG and of those the first one was remarkably reduced by a following flash given at 40 msec interval. This effect of paired flashes on the ERG was clearly reflected on the pre- and postsynaptic responses taken from the geniculate in which the preceding deflection failed to develop fully in response to the second flash as indicated by arrows. Under specified conditions such as high frequency of flicker or photopic adaptation, the response may be more pronounced by 'off' response. However, it is important to realize that under any condition behavioral features of retinal elements can be followed by geniculate neurons concerning at least rhythmic actions or phasic reactions.

Fig. 5. Effect of paired flashes on corneal ERG (upper beam) and the geniculate response (lower beam) under photopic condition (500 lux). Interval of flashes 49 msec. Arrows: negative going deflections. Stimulus artifacts on beams were exaggerated. Calibration: 200μV.



Discussion

The results in these experiments show that in rabbit the lateral geniculate response to a light flash exhibits marked oscillation which corresponds in time relation to that arising from the optic nerve or the retina. As described in the first section of this paper, typical response could be recorded from such relatively confined area as ventrorostral part of the dorsal neucleus. In view of the summed potential, this fact is ascribed to foregoing anatomical circumstances in rabbit's geniculate, but may depend in part on distribution of concerned elements within the neucleus. It is likely that such a rhythmic action is generated in some neurons which exert rather fast elapsing actions. In our unpublished study, we confirmed that 'on' or 'off' rhythms were conducted by large fiber groups of relatively high conduction rates. The result in the second section of this paper was the direct evidence on the geniculate response showing that each diphasic wave in the rhythm is preceded by an afferent incoming repetitively, and that a series of waves is not the self-sustained process which is initiated only by the first afferent. Finally, in the last section, the geniculate response was compared with the corneal or intraretinal ERG, showing that under various stimulus conditions behavioral features of the latter followed by the former. Doty and Kimura[5] found in monkey that similar rhythmic discharge

in the optic tract is apparently propagated to the striate cortex. Mitarai[6] has described on the after-discharge in the visual system of tod that it is generated in the retina and propagated up to the visual cortex. Thus, it appears that some part of oscillatory potential evoked in the retina is 'conductile' in nautre.

In order to produce rhythmic summed potential, it is necessary to maintain a synchrony of concernred unit response in any part examined. Such potential can be evoked in entire retina if a diffuse light is used as a stimulus, probably initiating at the bipolar cell layer, and may trigger repetitive firing of some ganglion cells at the same rhythm. In the latter case, those in the peripheral retina cannot contribute to the rhythmic summed potential arising from the optic nerve because of time delay which occurs during intraretinal propagation up to the optic disc. In other words, only limited elements distributed over the posterior part of the eye will have a driving effect on the oscillation in further proximal portions. In this respect, rabbit is available since it has majority of myelinated fibers in the retina. As mentioned above, in the optic nerve the oscillation seems to be propagated by fast fiber groups. However, temporal dispersion of impulses does occur to some extent in each burst on the way from the optic disc to the geniculate, resulting in slight attenuation of the rhythm. In spite of this, the geniculate response arises with marked oscillation which is comparable to the original pattern in the retina. It is rather surprising that frequency of the rhythm is hardly altered despite complicated processes intervening between them. Some of underlying synaptic mechanisms in the geniculate, facilitation or inhibition, would serve in part to rebuild the response pattern. Thus, reestablished information on evoked 'on' and 'off' rhythms will start in the lateral geniculate to the visual center. From our experiments on rabbits, we may conclude that similar rhythmic response can also be caused at the striate cortex.[9]

It may be reasonable to consider that the oscillatory potential under observation is a kind of phasic potential change initiating at the receptor site in the visual system, responding to an onset and a cessation of the stimulus light. We are mainly concerned here with 'on' rhythm because of difficulty of rabbit's retina in revealing distinct 'off' response under ordinary experimental conditions. However, similar rhythm of 'off' response was demonstrable in the optic nerve and the geniculate. Under specific conditions, as mentioned above, it was also recorded from the retina. Hence, that 'off' response is quite weak in rabbit's retina is not essential but is only a matter of quantity event. From this aspect, Nagata's[7] work on human ERG is of fundamental importance in showing that the oscillatory potentials occur responding to 'on' and 'off' of flickering light of various durations. On the other hand, as is generally known, there appear more slow or tonic potentials in response to an illumination, either excitatory or inhibitory in their processes and 'standing' or 'conductile' in their properties. Undoubtedly we may suppose that the channels are associated with such phasically

or tonically elapsing potentials in the visual pathway. The mechanism is not known with regard to the initiation of these rhythmical actions. Recently, Crapper and Noell[4] showed that a brief transretinal electrical stimulation can elicit a series of ganglion cell bursts which resemble the response pattern to a light flash. Baseing upon histological consideration, they discussed on the network subserving retinal inhibition with a conclusion that most probable site of action of the electrical pulse is the proximal end of the visual cells and that inhibition is evoked by interaction contacting receptor endings. Brown and Wiesel[3] found that discharge pattern concerning inhibitory or excitatory processe could also be recorded from bipolar cells as well as genglion cells. Thus, it is conceivable that the oscillatory potential may initially develop on the basis of specified interneuronal mechanisms which are localized around receptor terminals, involving bipolar cells or other adjacent elements.

Summary

1) In albino rabbit under urethane anesthesia an evoked summed potential to a xenon flash was recorded from the lateral geniculate body. Marked oscillatory potential could be recorded from ventrorostral part of the dorsal neucleus of the geniculate. Frequency or the period of this oscillation was hardly altered throughcut electrode penetration from the optic tract fiber layer to the lower margin of the neucleus.

2) Comparison was made on electrically and photically evoked responses with the same electrode position. A single shock of square pulse was applied to the distal end of the optic nerve, resulting in a response without any successive oscillation. In contrast with this, a photically evoked response was proved to be a series of waves of which each one was preceded by a small hump of afferent volley.

3) Under various stimulus conditions, changing of stimulus intensity or interval, behavioral features of the corneal or intraretinal ERG were followed by a geniculate response without remarkable difference in rhythmical actions.

4) The results led to a conclusion that the oscillatory potential may be a kind of phasic potential change which is initiated at the retina responding to 'on' and 'off' of a light stimulus, and some part of it may be 'conductile' in property being responsible for evoked rhythmic potentials in the visual pathway.

REFERENCES

1. Arden, G.B., and Liu, Y.M.: Acta Physiol. Scand., 48:36, 1960.
2. Bishop, P.O., Field, G., Hennesy, B.L., and Smity, J.R.: J. Neurophysiol., 21:529, 1958.
3. Brown, K.T. and Wiesel, T.W.: J. Physiol., 149:537, 1959.
4. Crapper, D.R., and Noell, W.K.: J. Neurophysiol., 26:924, 1963.
5. Doty, R.W. and Kimura, D.S.: J. Physiol., 168:205, 1963.
6. Mitarai, G.: Ann. Res. Inst. Env. Med. Nagoya Univ., 6:150, 1935.

7. Nagata, M.: Acta Soc. Ophth. Jap., 66:1614, 1962.
8. Yokoyama, M., Kaneko, K., and Nakai, Y.: Mie Med. J., 13:109, 1964.
9. Yokoyama, M., Taniguti, M., and Nakai, Y.: ISCERG, 4., 1965.
10. Yonemura, D.: Acta Soc. Ophth. Jap., 66:1566, 1962.

PATHOHISTOLOGICAL STUDIES ON MULTIPLE SCLEROSIS WITH ESPECIAL REFERENCE TO THE ARACHNOID AND BLOOD VESSELS IN THE BRAIN AND THE SPINAL CORD

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The etiology of multiple sclerosis is not yet definitely settled, but recently the view that this disease is due to allergic inflammation has come to be very influential.

Retrobulbar optic neuritis is said to be noted in about 50% of American and European cases of multiple sclerosis[9,12]; but in Japan multiple sclerosis is very rare in spite of relatively high incidence of retrobulbar optic neuritis.

In our clinic headed by Prof. Imachi about 500 cases of retrobulbar optic neuritis were craniotomized regardless of etiologic consideration, and in all of them chiasmal aranchnoiditis was clearly noted. And moreover, tearing and removal of the adhrent and thickned arachnoid membranes freed the cerebrospinal fluid circulation of mechanical obstruction, and brought about distinct improvements of vision and visual field. These clinical results have led us to the opinion that the investigation of the defence reaction in multiple sclerosis with especial reference to the mesenchymal system would contribute much to clarification of pathogensis of the disease. The present author, therefore, carefully examined pathohistological specimens of the brain and the spinal cord obtained from eight cases of multiple sclerosis. Arachonid-pial blood vessels and cerebrospinal intraparenchymal blood vessels were chiefly examined for inflammatory changes. Attention was also paid to perivascular cellular reaction.

Materials and Methods

Imachi, by courtesy of Assistant Prof. Haruo Okazaki, obtained formalin-fixed central nervous tissues of seven cases diagnosed clinically ane autopsically as multiple sclerosis at New York Stat University Hospital, U.S A.

These tissues were stained for microscopical examination after the following methods.

1. Hematoxylin-eosin stain.

2. Masson-Goldner's trichrome stain.
3. Akazaki's modified gitter fiber stain.
4. Sugamo's myelin sheath stain.
5. Heidenhein's myelin sheath stain[4].
6. Kultschitzky's myelin sheath stain.
7. Nissl stain.
8. Bielschowsky's silber stain, as modified by Suzuki.
9. Combined staning of myelin and glial fiber, as described by Lapham et al.[6]
10. Holzer's glial fiber stain.
11. Tsujiyama's modified glia stain.
12. Sudan III fat stain.
13. Periodic acid Schiff stain (PAS stain).
13. Weigert's elastic fiber stain.

Stained preparations from another case (Case 8) were cordially given to Imachi by Prof. Zimmerman of pathology, Montefiore Hospital, Columbia University, New York. These preparations consisted of specimens of myelin sheath stain taken from the pons, the cerebral cortex, the optic nerve, the optic chiasm, the medulla oblongata and the medial lemniscus and specimen of Nissl stain taken from the mid-brain. Table 1 gives salient clinical symptoms of eight cases, location of foci of demyelination, and areas examined in the present study.

Results

1) Arachnoiditis was common to all the cases. It was noted in almost all the areas microscopically examined (the frontal lobe cortex, the lateral ventricular inferior horn wall, the mid-brain, the medulla oblongata, the spinal cord, the optic tract, chiasm and nerve). Stratified hypertrophy and partial tubercular proliferation of endothelioid cell layers were noted in the arachnoid. Further findings were: swelling of arachnothels, marked proliferation and hypertrophy of connective tissues and trabecular cells, swollen degeneration and hyalinization of connective tissue fibers, diffuse lymphoid and histiocytic cell infiltration, and appearance of plasma cells in small numbers. Proliferation of argentophile fibers was generally mild. As to the spinal cord, these changes were present along its entire circumference.

2) The pia mater showed proliferation and hypertrophy of diverse degrees. Diffuse round cell infiltration, too, was noted in it.

3) With regard to the arachnoid-pial vascular system, small arteries showed adventitious hyperplasia, but were generally free from any serious medial and endothelial changes. In arterioles and precapillaries, however, both medial and enothelial tunicae were in a state of hypertrophy, and had suffered either hyalinization or fibrinoid degeneration. Many capil-

Table 1.

Cases	Sex	Age of Onset	Age of Death	Duration of Disease (Years)	Clinical Syntoms and Signs	Location of Demyelinating Lesions	Areas Examined
I	M	45	59	14	scanning speech, pallor of optic disc, stiffness of both lower extremities, abdominal reflex(−), Bbaniski Sign (+)	right superior frontal gyrus, paraventricular area, spinal cord	paraventricular area (posteior horn) spinal cord, medulla
II	F	22	38	16	nystagmus, abdominal reflex(+), dysuria, pallor of optic disc, ataxic gait, diplopia, paresis of r-upper extremity	brain stem, corpus callosum, left substantia nigra, cerebellum, right superior frontal gyrus, pons	optic nerve and chiasm
III	F	19	42	23	scanning speech, visual disturbance, temporal pallor of optic disc, intention tremor, inability of gait, nystagmus, incontinence of urine	lateral ventricular angle, mesencephalon, thalamus, cerebellum, pons	optic nerve and tract, area adjacent to III ventricle, medulla, pons
IV	F	30	65	35	weakness of both lower extremities	right calcarin cortex, tegmental pons, spinal cord, temporal horn of lateral ventricle	optic chiasm and tract, spinal cord, medulla
V	F	11	40	29	scanning speech, visual disturbance, pallor of optic disc, inability of gait, incontinence of urine, nystagmus, spastic paralysis of lower extremities	optic nerve chiasm, spinal cord, pons, white matter adjacent to lateral ventricles	area adjacent to lateral ventricle, optic, tract
VI	F	39	63	24	temporal pallor of disc, visual disturbance, abdlminal reflex, spastic paralysis of extemities	right temporal white matter, brain stem	area adjacent to lateral ventricle (inferior horn), optic nerve, chiasm and tract
VII	F	19	24	5	scanning speech, visual disturbance, simple atrophy of optic disc, nystagmus, spastic paralysis of extremities		frontal cortex, area adjacent to lateral ventricle and III ventricle, r-optic nerve, chiasm and tract
VIII	F	28	66	38			optic nerve, chiasm, pons, medulla

laries showed basiliar hypertrophy and adventitious hyperplasia. Endothelial hyalinization and cavity stenosis were also commonly noted in these small blood vessels. Perivenular regions were generally more or less severely infiltrated by round cells. In vascular walls, however, necrosis and large-scale cell infiltration were absent. Vascular cavity, too, showed no signs of thrombosis and occlusion.

4) In all the cases the arachnoid and the pia mater were observed to be in a state of inflammatory hypertrophy and proliferation. But there was no strict cor-relationship between inflammation and demyelination.

5) Demyelination characteristic of multiple sclerosis was noted in the cerebrospinal parenchym, and in the optic nerve, chiasm and tract. Foci of demyelination were irregularly sccattered in the white matter, being in various stages of development. Some of them had already reached the stage of sclerotic glial cicatrization, but others still remained young, and in them a large number of fatty granular cells were present. In demyelinated areas glial fibers had actively proliferated. Axis cylinder and nerve cell damage was very mild in contract with severe demyelination.

6) Some of demyelinated areas were clearly vasocentral. Small perivascular foci of demyelination existed together with large foci apparently formed by coalescence of small vasocentral foci.

7) As for vascular lesions in the brain and the spinal cord, and in the optic nerve, chiasm and tract, they consisted of such findings as fibrinoid degeneration and hyalinization of precapillary and capillary walls, endothelial hyperplasia and hyalinization, and cavity stenosis. They were not only sporadically located in the demyelinated regions, but present in milder forms in non-demyelinated regions as well. As in the case of the arachnoid, necrosis and intense cell infiltration were not demonstrated in vascular walls.

8) Perivascular cell reaction was generally very mild in the cerebrospinal parenchym, and in the optic nerve, chiasm and tract. In some cases, however, Virchow-Robin's cavity was distended and intensely infiltrated by cells. The infiltrating cells were mostly lymphoid and histiocytic.

9) The inflammatory nature of the disease was demonstrated by such findings as hemorrhages, vascular wall damage, arachnoid proliferation, degeneration of connective tissue fiber, vasocentral foci of demyelination, and intense perivascular cell infiltration in cerebral parenchymal foci of demyelination noted in some cases.

Discussion

Many laborious investigations have long been conducted on multiple sclerosis, but hitherto few descriptions of arachnoid lesions in this disease have been published. With a view

Fig. 1

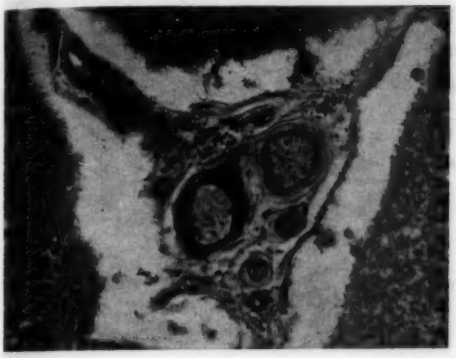

Fig. 2

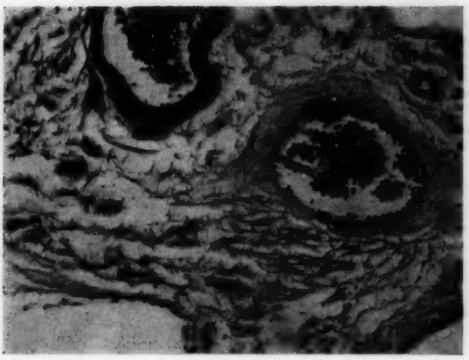

Fig. 3

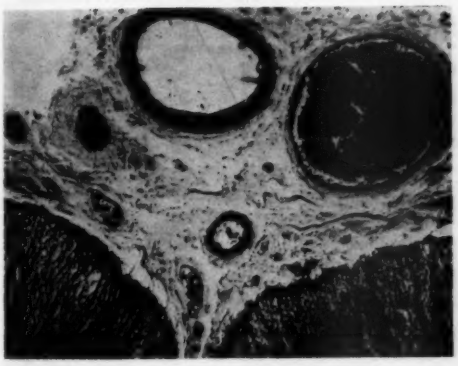

Fig. 4

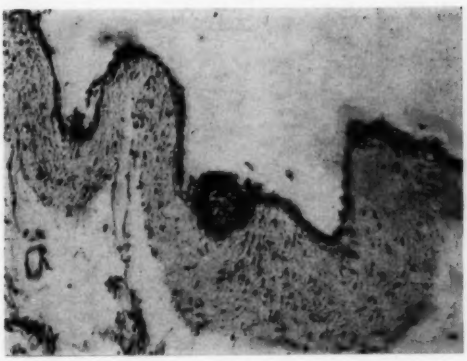

Fig. 5

Fig. 6

Fig. 7

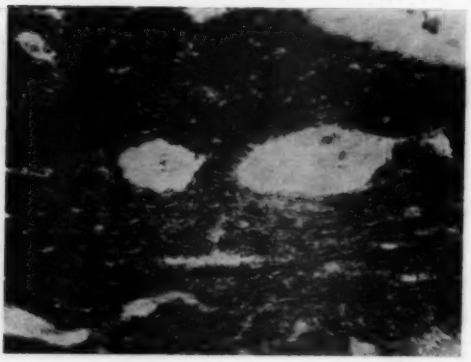

Fig. 8

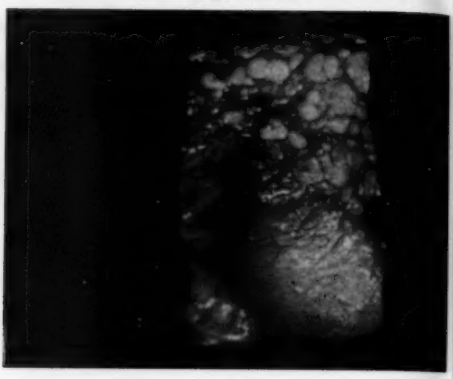

Fig. 9

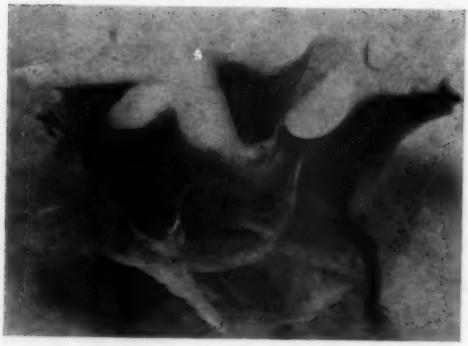

Fig. 10

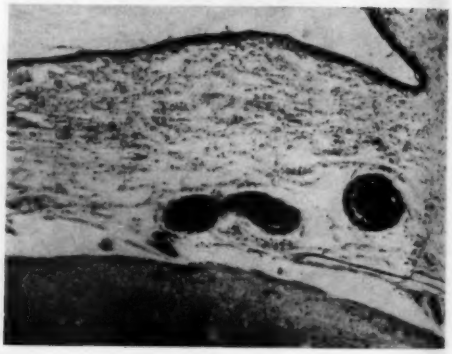

Fig. 11

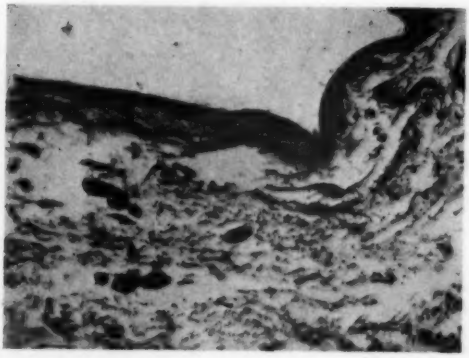

Fig. 12

to filling up this gap, the present author carefully studied arachnoid lesions in eight cases of multiple sclerosis whose diagnosis had been both clinically and autopsically established. She also confirmed the difignosis, noting that demyelination had occurred irregularly and sporadically mostly in the white matter, and that fresh and old demyelinated lesions existed side by side. Salient findings obtained from the arachnoid in the present investigation were as follows: round cell infiltration, proliferative reaction, hypertrophy and fibrinoid degeneration of small arterial walls, swollen degeneration and hyalinization of connective tissues, and education and tendency toward organization of fibrinoid. These pathohistological changes of the arachnoid, when coupled with cerebrospinal intraparenchymal damage (vascular wall damage, perivascular cell infiltration, hemorrhage and vasocentral demyelination), reveal a striking similarity between the pathohistological picture of multiple sclerosis and that of chronic allergy.

There has been some argument about the relation between arachnoid damage and demyelination in multiple sclerosis, but this argument may be reduced to the following three points.

1) Arachnoid damage comes first, and is followed by demyelination.

2) Factors responsible for demyelination are identical with those for arachnoid damage. Demyelination thus occurs simultaneously with arachnoid damage.

3) The arachnoid is secondarily affected after the commencement of demyelination.

The first of the above three hypothesis is refuted by the pathohistological findings. That is, arachnoid proliferative reaction was not so intense, while foci of demyelination showed highly advanced sclerotic cicatrization, and further, some parenchymal tissues remained normal and intact even in the areas adjacent to the arachnoid. It is unthinkable in view of these facts that demyelination is caused directly by spread of arachnoid damage, or indirectly by disturbance of the cerebrospinal fluid circulation.

The second hypothesis, if allergy is assumed to be the etiologic factor of the disease, seems very reasonable, according well with the pathohistological findings.

The third hypothesis is supported by Zimmerman, Netzky[14], and Hassin[8] who interprete arachnoid lesions as resulting from secondary, reactionary inflammation. This interpretation, however, does not fit in well with such findings as severe vascular damage, unrelatedness of arachnoid lesions to demyelination, intactness of ventricular epithelial cells around foci of demyelination, and cerebral intraparenchymal damage (vasocentral demyelination, vascular damage and cellular reaction).

Attention will now be turned to the cerebral parenchym. Demyelination was observed to be somehow related with blood vessels. This relation may be demonstrated by vasocentral occurrence of demyelination, formation of large foci by coalescence of small vasocentral foci, and the existence of small perivascular foci. Of course, there has been a long discussion on this

relation between demyelination and blood vessels. Roidin and Kolb[8] noted vasocentral occurrence of demyelination in a certain number of cases of multiple sclerosis. Adams and Kubik[1] recognized the existence of perivenous demyelination, and presumed large foci of demyelination to have been formed by coalescence of small vasocentral foci. The present author, obtaining similar findings, endorses this presumption.

As to vascular lesions, vascular walls generally showed hypertrophy, fibroid degeneration and hyalinization. This vascular wall damage might be regarded as a cause of demyelination, but as the same kind of damage was present in non-demyelinated areas as well, the conclusion on this point must be left for future investigations.

As to cellular reaction, very intense round cell infiltration was often noted in non-demyelinated areas. Zimmerman interpreted this kind of infiltration as absorptive reaction, but the above fact makes this interpretation untenable. Tokoro[10] also said that glial mesenchymal cell reaction in nearly undemyelinated areas often assumed too striking aspects to be considered as secondary absorptive inflammation. Under such circumstances allergy is the most adequate explanation for simultaneous occurrence of demyelination in the arachnoid and in the cerebrospinal parenchym.

Imachi's clinical experience with retrobulbar optic neuritis obliges the present author to attach special importance to arachnoid lesions in multiple sclerosis. In Imachi's opinion[5] what is called retrobulbar optic neuritis in Japan is a combination of optic parenchymal inflammation and degeneration with optic chiasmal arachnoditis. He found arachnoiditis in all of about 500 cases of retrobulbar optic neuritis of different etiology (toxicosis, Leber's disease, infection and vaccination) craniotomized by himself. Moreover, he succeeded in improving vision, visual field and central scotomata by removing the thickned and adherent arachnoid from around the optic nerve, and thus activating the cerebrospinal fluid circulation. The present author and her colleagues[7], too, once treated a case of Devic's disease and case of optic atrophy clinically diagnosed as multiple sclerosis. On craniotomizing these two cases, the author found their optic chiasmal arachnoid membranes thickned and adherent. Pathohistologically, too, the membranes were confirmed to be in a state of inflammation. In both cases removal of the damaged arachnoid effected a little improvement of vision. Vail[12], too, craniotomized a case clinically diagnosed as multiple sclerosis, and noted optic chiasmal arachnoiditis. He reported that loss of vision was prevented by removal of the damaged arachnoid, though the general condition of the patient went from bad to worse.

Having obtained such clinical and pathohistological results, the present author is convinced that arachnoid lesions, whether they may be regarded as primary affection, or as secondary reaction, will surely aggravate optic nerve disorders; and that inquiry into the true nature of arachnoiditis will contribute much to clarification of the pathogenesis of retrobulbar optic

neuritis and multiple sclerosis.

Summary

Careful pathohistological examination of the central nervous system of the cases clinically and autopsically diagnosed as multiple sclerosis revealed the following points.

1) More or less marked proliferative arachnoiditis and arachnoid-pial vascular damage were common to all the cases. No strict correlationship was observed between these pathological changes and demyelination.

2) Cerebrospinal intraparenchymal vascular wall damage (hypertrophy, fibroid degeneration, hyalinization and cavity stenosis) and cellular reaction were extremely strong in places.

3) Vascular wall and perivascular cellular reaction in the arachnoid, the pia mater and the cerebrospinal parenchym, and vasocentral demyelination unquestionably point to the inflammatory nature of the disease.

REFERENCES

1. Adams, R.D. & Kubik, G.S.: Amer. J. Med., 12:510, 1952.
2. Ferraro, A.: J. Neuropath. & Exper. Neurol., 17:278, 1958.
3. Hassin, G.B.: 144, New York, Paul B. Hoeber, 1922.
4. Izuka, R. & Tanishiki, T.: Psyatr. Neurol. Jap., 65:352, 1963.
5. Imachi, J. et al.: Acta Soc. Ophth. Jap., 67:1550, 1963.
6. Lapham. L.W. et al.: J. Neuropath. & Exper. Neurol. 23:156, 1964.
7. Nakagawa, A. & Ninomiya, Y.: J. J. Clin. Ophthal., 19:134, 1965.
8. Roidin & Kolb, L.C.: Advanced in Neurological Sciences, 2:666, 1958.
9. Schumacher, G.A.: New Engl. J. Med., 262:1069.
10. Tokoro, Y.: The Saishin-Igaku, 13:1057, 1958.
11. Uchimura, H., Shiraki, H. & Haruhara, C.: J. Neuropath. & Exper. Neurol., 16:139, 1957.
12. Vail, D.: Arch. Ophthal., 20:384, 1938.
13. Yaskin, J.C.: Amer. J. Ophthal., 34:687, 1951.
14. Zimmerman, H.M., Martin, G. & Netsky: Multiple Sclerosis and Demyelinated Disorder, 271, Baltimore, Williams & Wilkins' CO., 1950.

Fig. 1. (Case 1).
Blood vessels in the bulbar reticular lateral fascicular region.
Thickening, swelling, fibrinoid degeneration and hyalinization of arterioles and precapillaries. Venous wall hypertrophy. Basiliar thickening and cavity stenosis of capillaries. Lymphoid and histiocytic infiltratin in perivascular regions. PAS stain. 100×.
Fig. 2. (Case 1).
Arachnoid membrane (C_2 fissura mediana ventralis).
Proliferation of fibrous connective tissues; swollen degeneration of connective tissue fibers; venular adventitious hyperplasia and swollen degeneration. HE stain. 100×.
Fig. 3. (Case 1).
Arachnoid membrane (L_1 fissura mediana ventralis).
Proliferation of fibrous connective tissues; trabecular partial hyalinization; mild adventitious hyperplasia in spinalis ventralis, but no other marked damage. HE stain. 40×.
Fig. 4. (Case 3).

Arachnoid membrane (optic chiasm).
Marked hypertrophy and partial tubercular proliferation of endothelioid cell layers. HE stain 100×.
Fig. 5. (Case 4).
Right ventricular inferior horn wall.
Fibrinoid degeneration of vascular walls in demyelinated regions. PAS stain. 100×.
Fig. 6. (Case 5).
Ventricular inferior horn wall.
Intense round cell infiltration in demyelinated regions. HE stain. 100×.
Fig. 7. (Case 6).
The optic nerve.
Small-sized perivascular foci of demyelination in the neighborhood of large foci. Sugamo's myelin sheath stain. 40×.
Fig. 8. (Case 7).
Arachnoid membrane (frontal lobe).
Marked proliferation, turbidity and hypertrophy.
Fig. 9. (Case 7).
Arachnoid membrane (optic chiasm).
Intense fine fibrous proliferation and hypertrophy.
Fig. 10. (Case 7).
Arachnoid membrane (frontal lobe).
Marked proliferation and hypertrophy of endothelioid cell layers, connective tissue layers and trabecular tissue.
Fig. 11. (Case 7).
Arachnoid membrane (optic chiasm).
Irregular, marked hypertrophy and proliferation of endothelioid cell layers; proliferation and diffuse round cell infiltration of connective tissue layers and trabecular tissue; thickening of small vessle walls; basiliar thickening of capillaries. HE stain. 100×.
Fig. 12. (Case 7).
Arachnoid membrane (optic nerve).
Marked proliferation, hypertrophy and diffuse found cell infiltration of endothelioid cell layers, connective tissue layers and trabecular tissue. HE stain. 100×.

STUDIES ON PERSONALITY OF GLAUCOMA PATIENTS, ESPECIALLY ON THE YATABE-GUILFORD PERSONALITY TEST AND THE RORSCHACH TEST

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In recent years there has been an increasing emphasis on the study of psychosomatic medicine also in the area of ophthalmology. For instance, glaucoma, central angiospastic retinopathy, asthenopia and many other diseases are supposed to be classified in this category. The attitude which regards glaucoma as a psychosomatic disease belongs to a neuro-vascular school in a wide sense, only differentiated from the original theory in emphasizing the effect of emotions on the disease in its occurrence and course.

Emotional states, both acute and chronic, are closely related with personality patterns, and

their effects are considered to be transmitted to the effective organs by way of the autonomic nervous system and the endocrine organs.

Therefore, it becomes a very important matter whether the functional states of the autonomic nervous system and of the endocrine organs are normal or out of order.

Many efforts have been made to define the personality of glaucoma patients. In order to define the personality in question precisely, it is essential to examine adequate numbers of both congestive and open-angle glaucoma patients respectively by a projective technique like the Rorschach test and to compare them with proper control groups. However, no report has been presented yet so far.

In this report the writer examined personality and susceptibility of the autonomic nervous system of glaucoma patients, and tried to ascertain whether glaucoma could be a psychosomatic disease or not.

Methods

1. Personality tests.

A) The Yatabe-Guilford test, a modification of the Guilford personality test.

This test was applied to 20 normal persons (10 doctors and 10 nurses), 20 congestive glaucoma patients and 20 open-angle glaucoma patients. The test consists of 12 items and each item has 10 questions. The subjects were asked to reply 'yes', 'no', 'or' hard to answer.' Scores were calculated according to a definite rule.

B) The Rorschach test.

23 non-glaucomatous patients with chronic eye diseases (control group-Table 1), 20 congestive glaucoma patients (3 males and 17 females) and 20 open-angle glaucoma patients (10 males and 10 females) were examined by means of the original series of 10 ink blot designs. Normal persons were exempted from the control group so as to eliminate the personality factors which were thought to be common to all patients with serious eye symptoms and not peculiar to glaucoma patients.

Table 1. Non-glaucomatous patients (controls) in the Rorschach test.

Senile cataract	11	Phthisis bulbi	1
Retinal detachment	3	Traumatic cataract	1
Strabismus	2	Rodent ulcer	1
Uveitis	2	Herpetic keratitis	1
Traumatic vitreous hemorrhage	1	Total	23

From 23 controls 2 males and 13 females were selected at random (controls 1), and were compared with the congestive glaucoma group. In the same way, 10 males and 10 females were selected (controls 2) and were compared with the open-angle glaucoma group. It was to get similar ratio between the two sexes in either pair. As the Rorschach test findings are

strongly influenced by age and intelligence, the writer tried to gain, as nearly as possible, the same figures in age and educated period, of the four groups (Table 2). Finally, the tests were carried out by the writer alone.

Table 2. Age and educated period of controls and glaucoma patients who were examined with the Rorschach test. (mean and standard error).

	Controls 1	Controls 2	Cong. gl. patients	Open-angle gl. patients
Age	51.7±2.12	48.1±1.95	53.0±2.93	47.8±3.14
Educated period (years)	9.1±0.43	9.9±0.56	9.5±0.53	10.0±0.20

2. Functional tests of the autonomic nervous system.

A) On 9 cases of primary glaucoma, epinephrine and pilocarpine tests (pharmacodynamic tests) were performed.

B) The author examined by interview some subjective symptoms seen when the autonomic nerve system is out of order; namely, spleeplessness, fatigability, headache, excessive sweating, stiffness in the neck or shoulders, asthma, dyspnea, palpitation, dizziness, chilly constitution, diarrhoea, constipation, epigastric distress, nausea, heart burn, anorexia, and dysmenorrhoea. Two glaucoma groups were compared with non-glauconatous patients with chronic eye diseases.

Results

1. Personality tests.

A) The Yatabe-Guieford test.

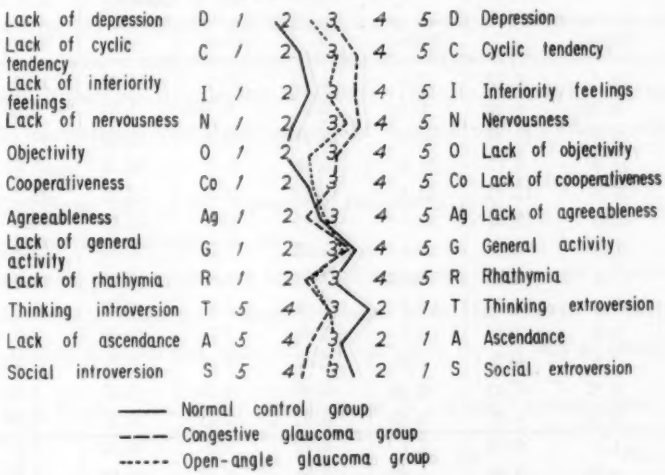
The average scores and the standard deviations of 12 times are shown in Table 3. Figure is the graphic illustration of Table 3. The congestive glaucoma group shows a tendency of so-called descending-to-the-left type, compared with the control group, which suggests social inadaptability. One of the open-angle glaucoma group occupies an intermediate position between the normal and congestive glaucoma groups. In comparison with the normal group, the congestive glaucoma group has higher scores on D, C, I, N, O, T, A and S, and has lower score on R; while the open-angle glaucoma group has higher scores on D, C, N, and T. These are statistically significant. The difference between the two glaucoma groups shows little significance except on S. (Table 4) and accordingly the both glaucoma groups are considered to have similar personality tendencies.

Summerizing the above data, it is inferred that glaucoma patients are generally depressive, anxious, meticulous, introverted, submissive and emotionally unstable, and that they have inferiority complex, difficulty in personal relations, and tendency to take subjective views on every matter.

Table 3. Yatabe-Guilford test findings.

	Normal controls (10 males and 10 females)		Cong. glaucoma patients (3 males and 17 females)		Open-angle glaucoma patients (11 males and 9 females)	
	Mean	σ	Mean	σ	Mean	σ
D	1.8	0.68	3.0	0.59	2.6	1.07
C	2.3	0.78	3.5	0.81	3.1	1.02
I	2.5	0.50	3.5	0.59	2.9	1.04
N	2.2	0.60	3.6	0.66	3.3	0.70
O	2.0	0.77	3.1	0.63	2.5	1.07
Co	2.5	0.97	3.0	0.74	2.6	0.97
Ag	2.7	0.91	2.4	0.66	2.8	0.99
G	3.5	0.87	3.3	0.77	3.2	0.93
R	3.0	0.84	2.4	0.96	2.5	0.81
T	2.2	0.87	3.1	0.44	3.1	1.00
A	2.8	0.60	3.5	0.97	3.0	1.26
S	2.6	0.83	3.7	0.84	3.1	0.89

Fig. 1. Graphic representation of Table 3.


Lack of depression
D / 2 3 4 5 D Depression
Lack of cyclic tendency
C / 2 3 4 5 C Cyclic tendency
Lack of inferiority feelings
I / 2 3 4 5 I Inferiority feelings
Lack of nervousness
N / 2 3 4 5 N Nervousness
Objectivity
O / 2 3 4 5 O Lack of objectivity
Cooperativeness
Co / 2 3 4 5 Co Lack of cooperativeness
Agreeableness
Ag / 2 3 4 5 Ag Lack of agreeableness
Lack of general activity
G / 2 3 4 5 G General activity
Lack of rhathymia
R / 2 3 4 5 R Rhathymia
Thinking introversion
T 5 4 3 2 1 T Thinking extroversion
Lack of ascendance
A 5 4 3 2 1 A Ascendance
Social introversion
S 5 4 3 2 1 S Social extroversion
Normal control group
Congestive glaucoma group
Open-angle glaucoma group

B) The Rorschach test.

The average scores and the standard deviations are summerized in Table 5. The congestive glaucoma cases have higher scores on Rej. T/R, T/R_1, and Dm%, and lower scores on R, Fc, R+%, P and BRS than the controls 1, while the open-angle glaucoma cases have higher scores on Rej, T/R and CF+C, and lower scores on M, R+%, P and BRS than the controls 2. These are statistically significant (Table 6). Moreover, with glaucoma patients CF+C is larger than FC, and ΣC is larger than M. It is noteworthy that comparison of the scores

Table 4. Levels of significance of differences in Yatabe-Guilford test findings.

	Differences between controls and cong. gl. patients	Differences between controls and open-angle gl. patients	Differences between two gl. groups
D	0.1%	2%	(—)
C	0.1%	2%	(—)
I	0.1%	(—)	(—)
N	0.1%	0.1%	(—)
O	0.1%	(—)	(—)
Co	(—)	(—)	(—)
Ag	(—)	(—)	(—)
G	(—)	(—)	(—)
R	5%	(—)	(—)
T	0.1%	1%	(—)
A	2%	(—)	(—)
S	0.1%	(—)	5%

Table 5. Rorschach test findings.

	Controls 1 (2 males and 13 females)		Controls 2 (10 males and 10 females)		Cong. glaucoma patients (3 males and 17 females)		Open-angle glaucoma patients (10 males and 10 females)	
	Mean	σ	Mean	σ	Mean	σ	Mean	σ
R	23.2	5.73	22.6	5.49	18.1	5.03	19.5	8.01
Rej	0.20	0.58	0.10	0.30	0.70	1.07	0.65	0.85
T/R	43.7	11.48	45.6	12.57	55.4	18.09	56.7	20.69
T/R_1	18.8	9.72	21.0	10.72	28.2	18.15	27.0	11.28
W%	37.6	12.22	38.6	14.23	33.0	13.91	42.1	19.67
D%	52.0	13.60	51.7	14.05	49.0	13.20	44.1	16.76
Dm%	10.4	4.33	9.5	4.28	17.9	8.72	13.8	10.51
S%	1.5	2.33	0.9	2.02	1.6	3.06	1.2	2.59
M	2.8	1.73	2.7	1.91	2.2	1.69	1.6	0.92
FM	4.8	2.26	5.2	2.18	3.7	2.68	3.9	1.91
m	1.2	0.91	1.0	0.88	0.82	0.71	0.73	0.90
FK	0.37	0.43	0.33	0.40	0.20	0.33	0.40	0.60
K	0.70	0.87	0.65	0.81	0.22	0.42	0.38	0.89
Fc	1.8	0.70	1.7	0.71	1.0	0.84	1.5	0.85
C′	0.80	0.98	0.80	0.92	0.45	0.59	0.98	0.81
FC	1.6	1.51	1.6	1.25	1.1	1.10	1.3	1.26
CF+C	1.6	1.17	1.3	1.17	1.9	1.25	2.2	1.30
ΣC	2.48	1.73	2.18	1.63	2.51	1.46	2.86	1.59
F%	41.9	12.51	42.6	11.48	46.5	17.25	44.5	13.22
A%	53.9	14.03	55.3	15.20	52.6	10.26	49.0	10.58
At%	2.5	3.32	3.3	4.76	4.1	6.11	6.2	5.95
R+%	76.3	2.97	71.9	9.44	67.0	10.72	65.4	7.68
P	6.0	1.09	5.8	1.36	4.4	1.06	4.5	1.50
BRS	+ 1.1	15.25	− 0.1	15.91	−13.3	13.74	− 8.5	10.24

Table 6. Levels of significance of differences in Rorschach test findings.

	Differences between controls 1 and cong. gl. patients	Differences between controls 2 and open-angle gl. patients	Differences between two glaucoma groups
R	2%	(−)	(−)
Rej	10%	2%	(−)
T/R	5%	5%	(−)
T/R_1	10%	(−)	(−)
Dm%	0.5%	(−)	(−)
M	(−)	5%	(−)
Fc	2%	(−)	(−)
CF+C	(−)	5%	(−)
R+%	1%	5%	(−)
P	0.1%	2%	(−)
BRS	1%	10%	(−)

between the two glaucoma groups has little statistical importance in all items. This was also recognized in the Yatabe-Guilford test findings. Whence the author thinks that glaucoma patients, both congestive and open-angle, have certainly the same trend in personality.

In summerizing the Rorschach test findings, it may be said that glaucoma patients appear to be meticulous, arbitrary and emotionaly labile, and to have perfectionism, obsessive idea, explosiveness and difficulties in personal contacts. The low levels of R+%, and of BRS will accelerate such tendencies. The above mentioned results are in accordance with the Yatabe-Guilford test findings.

2. The functional tests of the autonomic nervous system.

A) Epinephrine and pilocarpine tests.

All glaucoma patients showed abnormal reactions in the tests (Table 7).

B) Investigation of subjective complaints seen when this system is out of balance.

If the system is abnormal, there should naturally be this sort of complaints.

Table 7. Pharmacodynamic test findings of the autonomic nervous system.

			Ad. test	Pilo. test
T.H.	♀	acute cong. glaucoma	(−)	(+)
S.I.	♂	acute cong. glaucoma	(+)	(++)
J.O.	♂	chro. cong. glaucoma	(+)	(+)
T.O.	♀	chro. cong. glaucoma	(+)	(++)
T.H.	♂	open-angle glaucoma	(+)	(+)
K.Y	♂	open-angle glaucoma	(++)	(++)
B.A.	♂	open-angle glaucoma	(++)	(++)
N.S.	♀	open-angle glaucoma	(−)	(+)
H.I.	♀	open-angle glaucoma	(+)	(++)

It was ascertained that they had clearly more of these symptoms than the controls in many items (Table 8). Such tendencies are found to be stronger in the congestive glaucoma group. As to sleeplessness, stiffness in the neck or soulders, palpitation and dizziness, comparisons of the results drawn from the glaucoma patients and the controls by means of χ^2-tests are statistically significant (Table 9).

With regard to female cases the existence of dysmenorrhoea was also examined. (Table 10). Both congestive and open-angle glaucoma patients had many of these complaints compared with the controls: of those who had not any of these, there were only 7 cases (35%) in the congestive glaucoma group and 6 cases (50%) in the open-angle glaucomatics, while in the controls there were 11 cases (73%).

Table 8. Frequencies of subjective complaints seen when the autonomic nervous system are out of balance

	Controls (15 males and 15 females)		Cong. glaucoma patients (3 males and 20 females)		Open-angle glaucoma patients (10 males and 12 females)	
	No.	%	No.	%	No.	%
Sleeplessness	8	27	14	61	6	27
Easy fatigability	4	13	5	22	6	27
Headache	6	20	8	35	8	36
Exessive sweating	11	37	10	43	8	36
Stiffness in the neck or shoulders	9	30	18	78	13	59
Asthma	0	0	1	4	1	5
Dyspnoea	5	17	6	26	7	32
Palpitation	4	13	13	57	8	36
Dizziness	5	17	11	48	6	27
Chilly constitution	9	30	12	52	7	32
Diarrhoea	4	13	6	26	4	18
Constipation	10	33	9	39	6	27
Epigastric distress	4	13	3	13	3	14
Nausea	1	3	1	4	3	14
Heart burn	4	13	4	17	3	14
Anorexia	4	13	3	13	3	14

Table 9. Levels of significance of differences in frequencies of subjective complaints seen when the autonomic nerve is out of balance.

	Differences between controls and cong. gl. patients	Differences between controls and open-angle gl. patients
Sleeplessness	1%	(—)
Stiffness in the neck or shoulders	0.5%	10%
Palpitation	0.5%	(—)
Dizziness	5%	(—)

Table 10. Frequencies of dysmenorrhoea.

	Controls (15 cases)		Cong. glaucoma patients (20 cases)		Open-angle glaucoma patients (12 cases)	
	No.	%	No.	%	No.	%
Irregularity of menstruation	2	13	7	35	3	25
Hypermenorrhoea	1	7	6	30	3	25
Hypomenorrhoea	1	7	3	15	1	8
Bellyache or lumbar pain	1	7	9	45	3	25
No complaints	11	73	7	35	6	50

These results, together with those of pharmacodynamic tests, show that the faculty of the autonomic nerve is definitely abnormal.

Discussion

The importance of the psychic factors in glaucoma has been widely recognized by opthalmologists. Laquer[3], suffering from glaucoma himself, noticed the relations between environmental change and exacervation of glaucomatous symptom. The first attack occurred to him on the day when he was exhausted and depressed, subsided after mental diversion. It is recorded that he stated 'anger, anxiety, humiliation and even pleasurable excitement aroused by a stirring piece of music or a beautiful stage performance brought about an attack'. Inman[4] also cited a case of unconscious emotional distress appeared to precipitate attacks.

The writers examined precipitating factors of 16 cases of acute attacks and found close relations between the attacks and special emotional upsets at least in 8 out of these 16 cases. In this connection the effects of emotions on glaucoma attacks are indeed plain, but the question whether they have a definite influence on open-angle glaucoma course has not been solved yet. According to Posner[6] it is reported that no open-angle glaucoma but only narrow-angle glaucoma was influenced by such emotions. However it is evident from some reports by Schoenberg, Böhringer and other researchers that emotions have an important role even on open-angle glaucoma. We often come across such cases whose ocular tensions are high while they are outpatients but become low when they are admitted as inpatients. They sometimes puzzle us whether operations should be done or not. This may be a proof of emotional effect on open-angle glaucoma. The ultimate cause of glaucoma has not been known even today. It seems to be reasonable that the more an intimate relationship between glaucoma and emotion is clarified, the more a tendency to view glaucoma from a psychosomatic standpoint prevails.

For the study of psychosomatic disease it is said that the investigation of personality pattern is essential, because it has a close connection with individual personality whether a patient is apt to be influenced by emotions or not.

The fact had been recognized for years that the personality of glaucomatous patients is peculiar and we called it vaguely 'glaucoma character.' Hibbeler administered the Minesota Multiphasic Personality Inventory to white primary glaucoma patients (15 males and 12 females, including both chronic simple and acute cases).

She found that more than two third of the patients showed marked deviations on the personality scales, in striking contrast with only 5% of the normal group with such deviations, and that the trend of personality disturbance for the male patients was in the direction of 'depression' and 'hysteria', while the female patients toward 'paranoia' and 'schizophrenia'. Ripley and Wolff[8] investigated 18 cases of primary glaucoma by interview. They stated that in each case failure of adaptation was commonly evidenced by overconscientiousness and meticulousness, fluctuations in mood, excessive body preoccupation, difficulty in establishing and maintaining satisfactory personal relation, sexual maladjustment, and a multiplicity of other psychophysiologic disturbances as well as those of the eyes. Böhringer[9] examined personalities of 13 young glaucoma patients by interview and by the Rorschach test. He concluded that they were inclined to develope neurotic habit and showed such psychopathologic symptoms as depression, hypochondriasis, obsession, aversion, sexual disturbance and outstanding affectional instability. But he could not find a peculiar pattern of personality for glaucoma patients. According to his Rorschach test findings, there were the large figures of F+%, extratensive experience balances and many of shocks, rejections, shading, sexual and anatomic responses.

These results mentioned above basically agree with my observations. It is easily imagined that a person with such a personality disturbance fails in social life, suffering from a psychosomatic disorder in predisposed organs. Tsuji[10] states that patients suffering from psychosomatic diseases generally show numerous rejections and low scores of R, T/R and T/R_1 in the Rorschach test findings.

It is interesting to note that these characteristic findings are also observed in mine.

Now, how does personality disturbance or acute emotional upset precipitate a disorder?. The process is generally believed to be through neurohumoral pathway, and for this reason the susceptibility of the autonomic nerve and the functional state of the endocrine system has significance. The relation between ocular tension and autonomic nerve has long been emphasized, both clinically and experimentally.

There are many papers reporting that there often occur glaucoma attacks and elevations of ocular tension when the autonomic nerve system becomes out of balance, for instance, when in menstruation, over-exertion, low atmospheric pressure and so forth. As mentioned, emotional effects are also given rise to through the autonomic unbalance. Those who tested the functions of the autonomic nerve of glaucomatous patients found them almost abnormal

and they considered the unstability of the system as the cause of glaucoma.

There is only Ripley and Wolff's report[8] which examined subjective complaints concerning other organs of glaucoma patients. They said that in most cases other symptoms were found, such as precordial pain, palpitation, headache, urinary frequency, epigastric distress, diarrhoea, constipation and easy fatigability.

Personality, emotion and autonomic nerve are closely related to one another, and each is influenced by the other two. Ishigane[11] stated that those who had prominent anxiety, excitement and abnormal experience were inclined to sympathicotonia. Conversely, it is noted that autonomic unbalance causes emotional diturbance. Thus a vicious circle is formed. Whether this circle is strengthened or weakened depend upon personality pattern.

According to Abe[12], most of patients suffering from psychosomatic diseases show autonomic unbalance, and have complaints caused by its unbalance. Therefore, if this is true, results of my observation that glaucoma patients show nearly the same peculiarities support the idea which regards glaucoma as a psychosomatic disease.

Now there are also many reports about the endocrine function of glaucoma patients.

There is a mutual reciprocal relation between the autonomic nerve and the endocrine system, and unbalance of one disturbs another. As one of the tests about the endocrine system, the author[5] applied the Thorn's test to 30 primary glaucoma patients and gained the following results: that adrenocortical responses after injections of ACTH were intense in the acute congestive glaucomatics, while in the chronic, both congestive and open-angle, they decreased and even beyond normal limits in some cases. Numbers of blood eosinophils were few in early morning in acute glaucoma patients, but numerous in chronic glaucomatous patients. From these he concluded that adrenal cortical function was raised in the acute glaucoma patients and depressed in the chronic. Above mentioned dysmenorrhoea observed in female glaucoma cases may be partly referred to the disturbance of the endocrine system.

When glaucoma patients are in stress, they are presumed to response stronger than normal persons because of their emotionally labile personality and autonomic unbalance. Bodily changes in such a time are considered to occur mainly in circulator system, and if we reflect that production and drainage of aqueous humor are parts of circulation, stressors may well have an important role to play in occurrence and exacervation of glaucoma. This change occurs not in every eye in such a state but in anatomically predisposed eyes (organ selection).

Summary

1) The Yatabe-Guilford personality test was performed on 20 normal persons, 20 congestive and 20 open-angle glaucoma patients. In comparison with the normals, the congestive glaucoma patients had higher scores on D, C, I, N, O, T, A and S, and lower score on R,

while the open-angle glaucoma ones had higher scores on D, C, N and T. Differences of the results between the two glaucoma groups were not statistically observed except on S.

2) 23 non-glaucomatous patients with chronic eye disease (control group), 20 congestive glaucoma and 20 open-angle glaucoma patients were put to the Rorschach test.

The congestive glaucoma group had higher scores on Rej, T/R, T/R_1 and Dm%, and lower scores on R, Fc, R+%, P and BRS than the controls, while the open-angle glaucoma group had higher scores on Rej, T/R and CF+C, and lower scores on M, R+%, P and BRS than the control group. It is noteworthy that differences between the two glaucoma groups were not statistically significant in all items.

3) On the basis of these results, it may be assumed that both kinds of glaucoma patients should have the same personality trends, and that they are generally depressive, anxious, meticulous, introverted, submissive, arbitrary, and emotionally unstable, and have perfectionism, obsessive idea, inferiority feeling, difficulty in personal relations and tendency to take a subjective view on every matter.

4) On 9 cases of primary glaucoma, epinephrine and pilocarpine tests were performed as a functional test of the autonomic nervous system. In all cases abnormal results were obtained.

5) The author examined such subjective complaints as seen when the autonomic nerve system was out of balance. In comparison with non-glaucomatous patients, glaucoma patients had distinctly more of these complaints, especially, sleeplessness, stiffness in the neck or shoulders, palpitation and dizziness.

6) The fact that emotional experiences and other sorts of stressors take part in outbreaks and progressions of glaucoma provokes us to naturally consider glaucoma as a psychosomatic disorder. The writer is tempted to conclude that the above mentioned data of his support this idea.

REFERENCES

1. Harrington, D.O.: J.A.M.A. 133:669, 1947.
2. Kato, K.: Jap. Mon. Prak. Augenhk., 52:921, 1958.
3. Laquer, L.: Klin. Mbl. Augenhk., 47:639, 1909.
4. Inman, W.S.: Lancet, 2:1188, 1929.
5. Kato, M.: Acta Soc. Ophth. Jap., 69:1418, 1965.
6. Posner, A.: Eye, Ear, Nose, Throat, Monthly, 33:176, 1954.
7. Hibbeler, H.L.: Am. J. Ophth., 30:181, 1947.
8. Ripley, H.S. & Wolff, H.G.: Psychosom. Med., 12:215, 1950.
9. Böhringer, H.R.: Klin. Mbl. Augenhk., 123:283, 1953.
10. Tsuji, S.: Rorschachiana, Jap., 4:95, 1961.
11. Ishigane, M.: Psy. Neur. Jap., 60(9):59, 1958.
12. Abe, M.: Journ. Therapy, (Jap.), 46:1253, 1964.

Editional News

As reported previously in the editorial news of Vol. 9., No. 4, the proceedings of the fourth International Society for Clinical Electroretinography Symposium are under process by the editor and will be soon published under the auspices of J.J.O. Vol. 10 Supplement (approximately 400 pages).

This supplement will not be distributed without defraying its price which will be possible to report in the coming next issue.

The annual J.J.O. readers who want to subscribe this supplement will be able to obtain through Maruzen or other agents where usually distribute J.J.O. by subscription.

Other purchasers may order directly through the International Society for Clinical Electroretinography (ISCERG).

The Third Congress of the Asia-Pacific Academy of Ophthalmology will be held in Singapore during the first week of August, 1968, under the auspices of the Ophthalmic Department, General Hospital of Singapore.

The following announcement was made by Dr. Lim Kuang Hui, Organising Secretary of this Congress.

The Editorial Board sincerely wishes a successful Congress.

Preliminary Announcement and Invitation

The next 4-yearly Third Congress of the Asia-Pacific Academy of Ophthalmology will be held in Singapore during the first week of August, 1968.

The First Congress was held in Manila 1960 and the Second Congress in Melbourne, 1964.

For the Third Congress the scientific programme has been scheduled as follows:

(1) Major theme: "Prevention of Blindness in the Asia-Pacific Area with particular reference to Trachoma and Nutritional ocular conditions".

(2) Minor theme: "Recent advances in surgery with particular reference to management of ocular conditions in our region".

(3) Free papers.

(4) Films.

(5) Instrument Exhibition.

(6) Scientific Exhibition.

The language of the Congress in English.

The Congress Organising Committee invites:

(1) Films of a scientific or clinical problem of value to ophthalmology.

(2) Scientific papers of not longer than 20 minutes duration, followed by 10 minutes discussion.

Intention to participate in the Congress must be notified to the Organising Committee as early as possible. Film and scientific papers must be notified before September 30th 1967. Abstracts of not more that 200 words must reach the Committee before April 30th 1968 and the complete text before June 30th 1968.

For further information or for further notices please write to the Organising Secretary, c/o Ophthalmic Department, General Hospital, Singapore, 3.

Book Review

Biochemistry of the Retina: edt. Clive N Graymore, Academic Press, London & New York, 1965, 172 pages.

This book is published as a supplement to **Experimental Eye Research** and sums up all the proceedings of the 1st International Symposium, held in London last September.

Readers will be able to know today's tendency of retinal biochemistry by this book which comprises all interesting topics of this field. This book is also very helpful to search for the literatures about this.

There are contained various themes and consequently every report is brief. This fact makes easy to understand the focus of the topic.

A part of contents are as follows: session 1. fresh approach to rods and cones, molecular and kinetic properties of NAD and NADF linked dehydrogenases in the developing retina, etc., 2. studies on morphology, chemistry and function in isolated retina etc., 3. the problem of the retinal degeneration, 4. retinal metabolism by histochemistry, especially on Müller cell etc., 5. diabetic retinopathy etc.

As the fundamental biochemical works on retina were rather few recently, this symposium will give new impetus to consolidate the basis of the physiological study of the retina which are making a remarkable progress.

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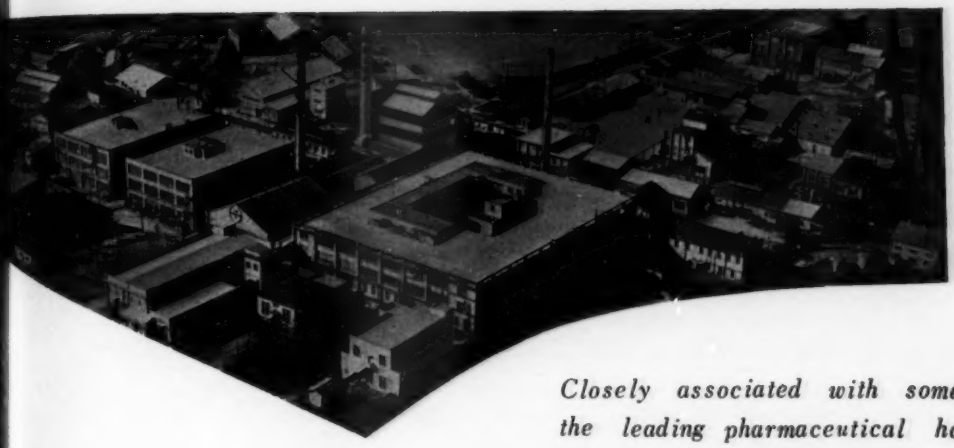

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